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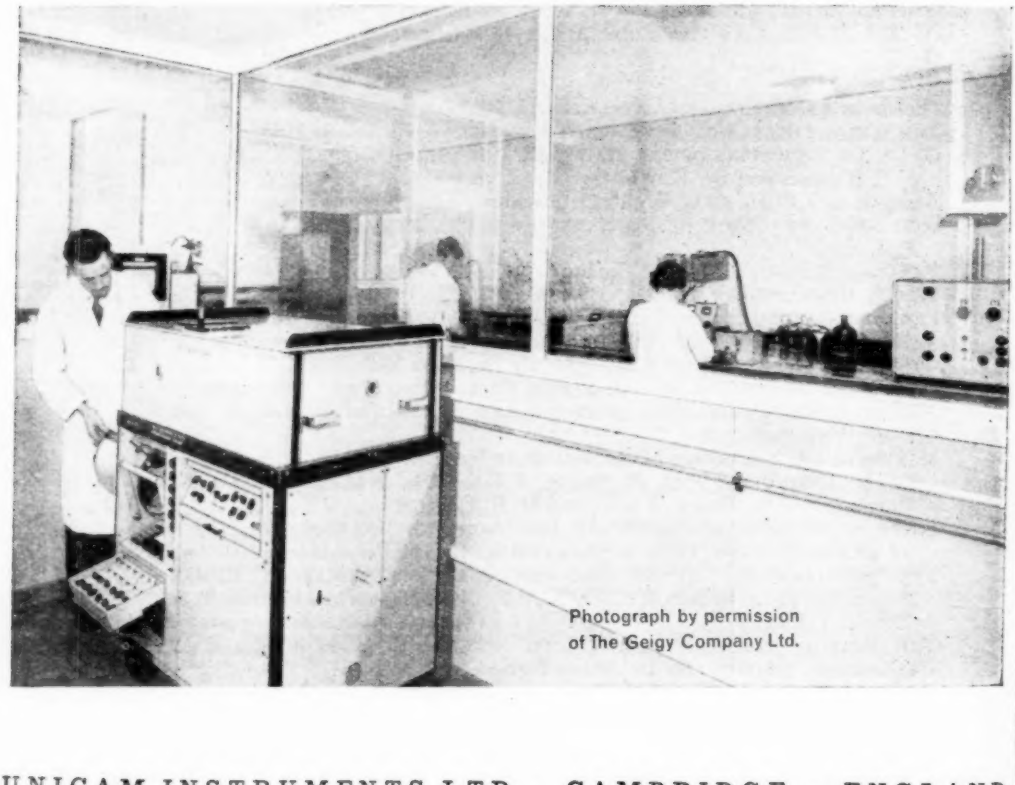

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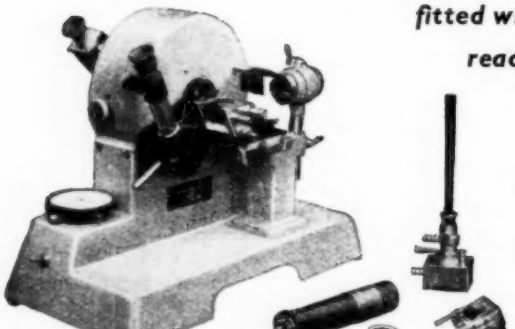

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CRISIS IN MATHEMATICAL EDUCATION IN BRITAIN

THERE is a strong possibility that mathematical education, as we have known it in Great Britain and in this century, will come to an end within twenty years, a possibility of incalculable consequences for the life of the nation as a whole. This warning, so often sounded by teachers of mathematics during the past decade and so often ignored, was given again with even greater urgency by Dr. Bryan Thwaites, the new professor of theoretical mechanics in the University of Southampton, in an inaugural lecture on May 23. With industry demanding and attracting more mathematicians, university teaching posts are left unfilled, and school teaching posts are unfilled or filled by those who have little interest in or knowledge of mathematics; thus there is a shortage of pupils having enough mathematical equipment to enable them to proceed to university or technical college training in science or technology. Hence, in the near future there will be a grave shortage of engineers and technologists, at a time when our export trade and our economic well-being will be more than ever dependent on the skill and availability of such people. Nothing less than measures on the largest scale, prosecuted with great vigour and determination, have any chance of restoring the position; such measures must be undertaken, not in some vague future, not in the next few years, but within the next few months. Even so, the schools would not feel any significant benefit before 1965 or 1966, and the universities and technical colleges not much before 1968 or 1970. While precise statistics are difficult to obtain, Thwaites's rough estimate serves to indicate the magnitude of the crisis. In the universities, the present staffing deficiency is equal to one year's Ph.D. output, and maintenance of staff at the present level requires double that output. In the grammar schools, the deficiency is equal to three years output of honours graduates, and maintenance requires again a doubling of output. This estimate takes no account of the needs of the newly founded or promised universities, of the technical colleges and of the secondary modern schools.

What measures does Prof. Thwaites propose? First he suggests that the mathematicians should set their own house in order. There must be a greater sense of unity among all types of mathematician, with the formation of a professional institute to which all would naturally belong; he regrets that the Mathematical Association does not have a standing comparable with that of other recognized professional bodies. Then the dichotomy between teaching and research must be bridged. It should not be axiomatic that the only career for a first-class honours student is in research; the appointment and promotion of a lecturer should not depend solely on his success in getting his name into print. Here Prof. Thwaites perhaps fails to do justice to the Mathematical Association; it has never claimed or wished to be concerned with anything except the promotion of good methods of teaching mathematics. The London Mathematical Society caters for the research worker, and Prof. Thwaites might have reinforced his argument by deploring the recent tendency for a mathematician to join either the Mathematical Association or the London Mathematical Society but not both. Further, the Mathematical Association has made a significant contribution towards easing the crisis by establishing a diploma in mathematics; by passing this, those teachers who, with no specialized mathematical training, are being drafted into teaching mathematics, may equip themselves with at least the basic knowledge which they will require.

Prof. Thwaites then looks at the outside aspect of the problem, and divides it into two parts: the problem of recovery, and the problem of maintenance. For recovery, demands for mathematicians made by other professions must be voluntarily and severely contracted; salary scales for teachers must be interpreted with great flexibility; interchange between the school and the university on one hand, and industrial, commercial and Government establishments on the other, should be widespread, on a part-time or full-time basis. This programme must have national acceptance, and must therefore be presented as an urgent national necessity by a fully organized publicity campaign. For maintenance, we must double the mathematical entry over the whole of Britain at the earliest possible moment. Since we have not enough teachers to cope with the present entry, this would appear to be an impossible demand. But Prof. Thwaites thinks that much could be done if the syllabus for school mathematics could be reformed, if the universities revised their standards of entry, and if more undergraduates could be encouraged to take the unduly disparaged general degree in which mathematics would be read in conjunction with other subjects. Here resolute leadership from the centre is essential.

Already, this lecture has excited much comment. Questions have been asked in Parliament (see p. 127 of this issue). In the *New Scientist* of June 1, Prof. C. A. Coulson and Dr. J. M. Hammersley have directed attention to this bottleneck in British science and technology. They note the success of the National Science Foundation of the United States, set up by the Federal Government in 1950, to provide courses for the further training of practising teachers, and to establish groups charged with the revision of the school curriculum and with writing of new text-books. In particular, they stress the value of the summer institutes, which provide genuine refresher and train-

ing courses lasting 6 or 8 weeks. Letters from leading mathematicians to *The Times* have welcomed some of Prof. Thwaites's proposals, have been severely critical of others, and have suggested alternatives; but none has impugned his statement of the magnitude and urgency of this problem. There is fairly general agreement that an effort must be made to force the country to understand that a shortage of mathematics teachers is not a small, sectional matter, but the symptom of a coming national catastrophe, that, in fact, we may already have passed the point of no return. Although various bodies have seen the danger and have issued warnings, none of them has so far been able to command the attention of the nation as a whole.

If the danger is great—and no one has yet suggested that it is not—then the responsibility for meeting it lies not only on the teaching and research associations, but also on the University Grants Committee, the Ministry of Education and the Ministry for Science. Action at Cabinet-level may be needed; but a fact-finding committee would be useless. There is no time for a minute inquiry into minor statistical detail, and no salvation in a report that the problem is insoluble and so may safely be left to solve itself. A small strong committee, taking for its motto Florence Nightingale's words, "Reports are not self-executive", might yet retrieve the situation.

WAVE PROPAGATION AND GROUP VELOCITY

Wave Propagation and Group Velocity

By Léon Brillouin. (Pure and Applied Physics: a Series of Monographs, Vol. 8.) Pp. xi+154. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 6 dollars.

IT is difficult to see what purpose this book is meant to serve. Ostensibly it is to make available certain original papers which are not easily accessible because they were published in Germany in 1914. In his foreword, Dr. Brillouin says: "All these modern developments made it advisable to assemble here a systematic presentation of the original papers, which are rather difficult to find nowadays. It is hoped that the present book will be helpful to many readers and save them time and trouble, especially the trouble of recomputing and rediscovering many important features of the general theory". And again in the preface: "I discussed the solution in great detail and gave a complete answer. . . . These problems have come again into the foreground in connection with radio signals and radar. Reflection in the Heaviside layer requires a real knowledge of all these definitions (of group velocity)". But the "modern developments" referred to are in fact very old history now. Of more than sixty references given to the literature only seven are later than 1930, and of these only three are really relevant. The latest reference to propagation in the Heaviside layer is 1931.

One gets the impression that in Dr. Brillouin's view "les jeunes" have much to learn and little to teach concerning the propagation of waves, and that if they will just take the trouble to read this little book they will find all that is important about the subject. This cannot be accepted. The papers are badly written, and they do not by any means give the "complete answer" even to the limited problem with which they deal.

This is not to say that the book lacks interest. The subject is in fact a fascinating one. When a nearly monochromatic light wave with a sharp front falls on a transparent medium the disturbance that travels through the medium consists of three parts: a first forerunner, travelling with the same speed as light *in vacuo*; a second forerunner; and then the main signal, travelling with a velocity which is, except near regions of anomalous dispersion, the group velocity.

The forerunners have a very small amplitude in comparison with the main signal. The first forerunner has a very high frequency. If the incident signal rises from zero to a steady sine wave suddenly, then the frequency of the first forerunner is at first much higher than the frequency of the light, and then gets progressively less, the amplitude meanwhile increasing. But if the incident signal is not so idealized, this rapidly oscillating aspect of the first forerunner is blurred. The second forerunners are of lower frequency, comparable with the signal frequency and their onset travels with a velocity characteristic of the medium (and less than the velocity of light *in vacuo*). They then increase rapidly in amplitude as the main signal arrives, and merge with it.

These deductions from the electromagnetic theory of matter show that no signal travels faster than vacuum light velocity. The first forerunners travel with exactly that velocity. There is, of course, no mystery here. The medium is supposed to consist of electrical particles which on average are at rest before the signal arrives. When it comes, the particles take a little while to get moving, and so to modify the primary field, which, of course, is travelling with vacuum light velocity in the empty space between the electrical constituents of matter.

The book looks at this problem from several slightly different points of view, bringing forward some interesting considerations, in particular the idea of velocity of energy propagation, as distinct from signal velocity, and group velocity. These three velocities are for all practical purposes the same except in regions of anomalous dispersion, where strong absorption of the signal confuses the picture. Unfortunately, much of the value of this discussion is marred by the repetitive nature of the chapters. Had the subject been treated in a unified manner, instead of a 're-hash' of several published papers, the book could have been shorter and very much clearer. There is a deal of inelegant mathematical detail, particularly in Chapter 3, which one presumes to have been Dr. Brillouin's doctor's thesis under Sommerfeld. By contrast, Chapter 2, which is a survey of the subject by Sommerfeld himself, is a masterpiece of clarity and style. It is a pity that Dr. Brillouin did not rewrite the rest of the book nearer to this model. The final chapter is an odd one, having really very little to do with the rest of the book. It deals in a rather cursory manner with wave-guide propagation and looks strangely out of place.

The translation into English from the German and French of the originals is inept. The figures are difficult to follow because the labelling of different curves is not clearly distinguished in the legends. There are many misprints.

M. H. L. Pryce

THE CYCLOL REVIVED

Chemical Aspects of the Structure of Small Peptides
An Introduction. By Dr. Dorothy Wrinch. Pp. viii+194. (Copenhagen: Ejnar Munksgaard, 1960.) 3.50 dollars.

REVIEWING the chemistry of the proteins in 1937, D. Jordan-Lloyd wrote "the chemistry of the proteins is a mystery no longer", and this enthusiastic overstatement was not out of touch with much informed opinion at that time. The prevailing optimism of the late 'thirties concerning our understanding of protein chemistry was, to a large extent, based on two newly postulated general hypotheses; these were the Bergmann–Niemann periodicity hypothesis and Dr. D. Wrinch's cyclol hypothesis. Although neither hypothesis was ever completely accepted, the former, indeed, being later conclusively disproved, both were of crucial significance in the development of protein chemistry, leading as they did to a renewed interest in the subject and to much important experimental work; for this reason their proponents will always occupy an honoured position in the history of protein chemistry. Very recently, something like the Bergmann–Niemann hypothesis, in much modified form, has been revived by Prof. Šorm, while, in the present book, Dr. Wrinch modifies the cyclol hypothesis and extends it to small peptides.

It is most unlikely that the views now advanced by Dr. Wrinch will command widespread acceptance; the reason for this is fundamentally semantic. Dr. Wrinch starts from the undoubted fact that in certain compounds containing amino-acid residues, such as the ergot alkaloids and the aspartyl-lysine 'dipeptide' from bacitracin *A*, these residues are not combined through simple amide linkages. She then argues that the existence of such compounds "means the repudiation of the peptide hypothesis, for if a hypothesis is subject to unpredictable exceptions, no predictions from it are reliable", and proceeds to a detailed examination of all the possible ways in which amino-acid residues might be combined, arriving finally at a generalized structural theory in which the 'cyclol' and 'amide' structures figure as special cases. Such an approach is, however, mathematical rather than chemical and will not be accepted by organic chemists. For an organic chemist, a peptide is defined as a compound made up of amino-acid residues joined head-to-tail by amide linkages, and the exceptional compounds cited by Dr. Wrinch are, quite simply, not peptides; Dr. Wrinch, on the other hand, appears to define a peptide as a compound made up of amino-acid residues, without restriction as to the manner in which these residues are joined together. In her earlier writings, Dr. Wrinch posed the question "Are proteins peptides?" and gave a possible answer to this meaningful question; now, however, she asks "Are peptides peptides?" and most organic chemists will find this a meaningless question.

In spite of these objections to her main thesis, Dr. Wrinch's new study is both significant and valuable. It is certainly possible, and some think probable, that the active centres of enzymes and other biologically active proteins involve some chemical modification of the simple peptide chain; Dr. Wrinch's systematic classification of such modifications will be of real value in stimulating further thought on this important topic. All organic chemists and biochemists interested in biologically active proteins should read this little book for, although they will probably disagree with its conclusions, they will derive from it much stimulation.

H. N. RYDON

OPERATIONS RESEARCH AND SYSTEMS ENGINEERING

Operations Research and Systems Engineering
By Charles D. Flagle, William H. Huggins and Robert H. Roy. Pp. x+889. (Baltimore, Md.: The Johns Hopkins Press; London: Oxford University Press, 1960.) 116*s*. net.

THIS book consists of a collection of the lectures given in the annual two-week course for management at the Johns Hopkins University, under the same title as the book. The lectures came from various academic departments and also the Applied Physics Laboratory and the Operations Research Office. They will be useful to the student entering on a career in operations research or industrial engineering, and also to directors, managers, and men of business generally who want to know what operations research is about, how it works, and what are its methods and achievements.

There are three parts, the first dealing with philosophical, historical, social and psychological aspects of the subjects. Some of the lectures, considering their length, are rather thinly spread with ideas of significance or importance; and it would surely be possible to say as much with fewer words. However, the lecture by Ellis A. Johnson, on "Operations Research in the World Crisis in Science and Technology", is a most important exposition and assessment of some of the great global forces which are shaping the future of mankind. The second part (more than half of the book) deals with methods and includes accounts of models, statistics, computers inventory, programming, queueing, simulation, design of experiments, information theory, system dynamics, and feed-back and stability. It must be stated that while each lecture is informative the accounts are only introductory. For example, the chapter on queueing theory gives an admirable account of what the subject is about and what it can do, but it does not go very much into mathematical methods. The third part, possibly the most valuable section of the book, is concerned with case studies. Three of these are industrial and concern the functioning of a hospital, a newspaper firm, and a telephone company. For the last-named it is the cost and value of reports which are studied. All three present thorough investigations and reveal the complex structure of practical operations research problems.

The subjects of operations research and systems engineering have developed rapidly in recent years, and the book under review demonstrates conclusively that what has been developed is not just a fashion but something much more permanent. The new way of looking at the operation of the total system, consisting of men and machines, has come to stay—at least in a society concerned with the reduction of waste, the improvement of efficiency, and progress towards a generally higher standard of living. To anyone concerned with this new point of view the book may be thoroughly recommended.

L. S. GODDARD

XUM

COCKROACH ECOLOGY

The Biotic Associations of Cockroaches

By Louis M. Roth and Edwin R. Willis. (Smithsonian Miscellaneous Collections, Vol. 141, whole volume). Pp. vi+470+36 plates. (Publication 4422.) (Washington, D.C.: Smithsonian Institution. 1960.) n.p.

DURING the past decade the researches of Drs. Roth and Willis have contributed very greatly to our knowledge of the biology and behaviour of cockroaches, particularly those of economic importance. Not only have they published the results of much painstaking original work, but they have also brought together in the form of comprehensive compilations the fruits of the work of hundreds of other workers in this field. The present work is a compilation of this kind and its bulk is an indication of the thoroughness with which the authors have undertaken their task.

As the title of the book suggests, the authors are primarily concerned with the animals and plants associated with cockroaches; but, as a chapter of some eighty pages is devoted to the physical environment of these insects, the compilation may fairly be said to cover the whole subject of cockroach ecology. In classifying the cockroach's associates the authors begin, commendably, by defining their use of the words symbiosis, mutualism, commensalism, parasitism, and predatism. They then set out a comprehensive biological classification with page references to the account of each category in the text.

After a brief chapter on mutualism, most of the remaining plants and animals associated with cockroaches are dealt with group by group; they are classified down to the level of family and are then listed alphabetically under the generic name. The cockroaches associated with each organism are listed as natural or experimental hosts, vectors, or prey. Then follows a most useful check-list of the naturally occurring symbiotic associates of cockroaches arranged alphabetically under the cockroaches concerned, with page references to the preceding classified section of the book. The remaining few short chapters deal with such topics as commensal and predatory cockroaches, associations among cockroaches, and the biological control of cockroaches.

The bibliography covers more than eighty pages and comprises some fifteen hundred entries; it must include almost every publication having any bearing on cockroach ecology that has appeared up to the end of 1959, and certainly represents the first attempt to bring together the vast literature on this subject.

There are a few text-figures, mostly of Protozoa, and thirty-six half-tone plates showing some excellent photographs of living cockroaches and some of the more important organisms associated with them. These plates include photographs of both sexes of all the major cockroach pests and are a most valuable supplement to the book.

This compilation has achieved a very high standard and is beyond criticism in all important respects. There are, however, a few minor instances of inconsistency: English common names, for example, are given for many but not all organisms that have them, and dates—in addition to authors—are cited for the specific names of helminths, but not for those of any other group (these dates serve no useful purpose in a book of this type and might well have been universally omitted). In the Contents the page references from p. 308 onwards are all out by two pages.

It may be said in conclusion that this book can be unreservedly recommended to any worker on cockroach biology, and particularly to those concerned with the economic importance of these insects. Together with the two previous monographs by the same authors on the reproduction of cockroaches (1954) and their medical and veterinary importance (1957), it forms a most worthy contribution to the literature of cockroaches. DAVID R. RAGGE

TESTING TEXTILES

Handbook of Textile Testing and Quality Control

By Prof. Elliot B. Grover and Prof. D. S. Hamby. Pp. vi+614. (New York: Textile Book Publishers, Inc.; London: Interscience Publishers, Ltd., 1960.) 132*s*.

THE control techniques used in laboratories in the textile industries have increased enormously in range in recent years and become more widely and intensively applied. Despite this, text-books on the subject are much as they were ten years ago, with most students having to rely on the *British Standards Handbook* No. 11, which is not a text-book about principles but a series of sets of instructions commended for doing certain tests chosen for their reproducibility. The appearance of Grover and Hamby's book is therefore especially welcome in that it does describe up-to-date instrumental techniques in regular use in the United States.

More than a hundred pages are given to statistical methods, vital in interpreting test results on textile materials and also in the theory of yarn structure. Another hundred pages are devoted to tests on cotton fibres for length, strength, fineness and maturity and other properties. Considerable space is given to yarn tests, including the most up-to-date tests for irregularity and strength. Methods of test for fabric receive less attention, though a chapter on the evaluation of fabric defects is valuable.

The book is thus concerned with physical testing only—methods of fibre identification, or chemical analysis of blends, or estimation of damage or degree of polymerization are not mentioned, nor, oddly enough, are the highly developed methods of test for the abrasion resistance, crease resistance and water repellency of fabrics. The book is strongly oriented towards cotton, and neither wool nor synthetic fibres receive much attention, although much has been published on test methods appropriate to them.

The treatment of the selected subject-matter is thorough but elementary, perhaps too elementary in some parts. An honest attempt has been made to present some difficult concepts in simple terms—as in the chapters on statistical methods and yarn evenness—but parts of the book are taken up with instructions for operating particular instruments that would have been better left to the makers' handbooks. Whether the tests measure the right parameters, their limits of accuracy, the reproducibility of tests made on different instruments or by different operators, their relative convenience—these are matters that might have been given more space in a book on quality control. The bibliographies at the end of each chapter are generally

unsatisfactory and should be thoroughly overhauled in any reprinting—some have no bibliography at all, sometimes an author is listed but with no journal or date, sometimes a reference made to unpublished work appears alone, and practically all the lists are absurdly short. The manner of presentation leads one to speculate about the kind of reader envisaged, and to the conclusion that the American executives, technical personnel and students, for whom this is written, are no better trained in science than their English counterparts.

W. J. ONIONS

A CATALOGUE OF THE ULTRA-VIOLET

Organic Electronic Spectral Data

Vol. 1 : 1946–1952. Edited by Mortimer J. Kamlet. Pp. xiv+1208. 215*s*. Vol. 2 : 1953–1955. Edited by Herbert E. Ungnade. Pp. x+919. 132*s*. (New York : Interscience Publishers, Inc. ; London : Interscience Publishers, Ltd., 1960.)

THE object of this work is to present all useful published data on the ultra-violet and visible absorptivity of organic compounds. The criterion of usefulness is adequacy to define the molar absorptivity for at least one stated wave-length of maximum absorption. The solvent used, or *p*H of an aqueous solvent, is also stated, if known, though the concentration is not ; and literature references are given for all entries. Compounds are arranged as a molecular formula index, and the number comprised must be between twenty and thirty thousand, for many of which more than one set of data is supplied. Gas-phase measurements are included.

Critical treatment of this material, difficult in view of its great bulk, becomes nearly impossible because of the chaos of modes in which the data have originally been expressed. The editors give candid warning that, despite their best endeavours, a proportion of the figures may thus be out by a factor of ten, or of the molecular weight, or both, and that the reference to the original paper should be followed up in any case of doubt. Within this limitation, absorptions are here uniformly expressed as molar absorptivity, log ε, where ε has the value $\log(I_0/I)/cb$, c being in cm. and b in moles/l. The latter expression is now adopted as the definition of 'extinction coefficient' preferred by the Optical Data Commission of the International Union of Pure and Applied Chemistry. A statement of only two significant figures of the mantissa, however, can be appreciably less precise than the measurement it reports. Thus a figure of 4·23, signifying the range 4·225–4·234, would cover (for 0·001 per cent of a solute of molecular weight 300) measured extinctions from 0·559 to 0·571. For quantitative purposes, significance in the third figure is desirable and attainable, and it seems matter for some regret that, in cases where such precision has been recorded, it must be lost in the editorial reduction to two figures.

The search for material in more than seventy journals appears to have been extremely thorough. Other sources—to judge by the omission of, for example, amodiaquine (*British Pharmaceutical Codex*, 1954), chlorpheniramine (*U.S. Pharmacopoeia*, 1955) and the chlorosalicylanilides (*American Medical Association's Standards for New and Non-official Remedies*, 1955)—have not been drawn on.

The choice between systematic and coined names is not always made consistently; for example, "Antazoline" is used, but not "chloroquine" (*U.S. Pharmacopoeia*, 1955, and *Chemical Abstracts Index*, 1954), though the latter is an example where the coined name would make for easier reference among the seven entries under its molecular formula.

The numerals in the formulæ of Volume 2 are smaller but sharper than in Volume 1 ; a combination of the best qualities of both would seem ideal. That a work of such enormous scope should realize everyone's ideal in every respect, however, is scarcely conceivable ; and the measure achieved here, and promised in the further volumes by which the project will be brought up to date, must claim the gratitude and respect of all users of data of this kind.

E. G. KELLETT

EUROPEAN PARASITES OF MAN

Parasitologie für Ärzte

Von Prof. Otto Jírovec. Unter Mitarbeit von Dr. J. Jíra, Dr. E. Kmetý, Dr. J. Kramář, Dr. M. Petrů und Dr. B. Rosicky. Pp. xi+684. (Jena : Gustav Fischer Verlag, 1960.) 93.65 D.M.

THERE are, Dr. Jírovec says in his preface, several good books on medical parasitology ; but most of these deal more especially with parasites found in man in tropical and subtropical countries and few concentrate on species found in European climates. This book, however, gives more of its space to these, the tropical and subtropical species being relatively briefly described. The emphasis throughout is on bionomics, epidemiology, diagnosis and therapy, less space being given to the clinical signs of parasitic disease.

The chief author, Dr. Jírovec, has written the general account of parasitism (Part 1), the description of parasitological techniques (Part 2) and the sections on the Protozoa (Part 3) and helminthology (Part 4). The section on the Protozoa occupies almost half the book and reflects, in considerable detail, the work of Dr. Jírovec and his colleagues, and many will be glad to have the accounts here given of parasites such as *Pneumocystis*, *Toxoplasma* and *Trichomonas vaginalis*, which are less extensively discussed in other text-books. The section on the arthropods (Part 5), on parts of which Dr. Rosický collaborated, is on the whole less evenly covered. The section on the leptospiroses was written by Dr. Kmetý, the account of *Toxoplasma* by Dr. Jírovec in collaboration with Dr. Jíra and the sections on the rickettsias, *Trichomonas vaginalis* and *Pneumocystis* were revised by Prof. Vaněk, Prof. Peter and Dr. Syrůček respectively. Other collaborators were Dr. Kramář on the Diptera and Dr. Petrů on paragonimiasis and ascariasis of the lung.

The book, which is written in clear and simple German, fully maintains the high standard of production of the house of Gustav Fischer. The 285 illustrations are all good and many of them, supplied by the author or his colleagues, are not available in other books. They include useful photographs of helminth eggs and of parasites in the host's tissues, and some of these were taken through the phase-contrast microscope. There are several illustrations in colour, and, although most of these will be helpful to the reader, some of them raise the question, also raised by coloured illustrations in other text-books, whether

colour, especially when it depends on the staining techniques employed, is worth its high cost. The reproduction of all the illustrations is, no doubt, improved by the use of glazed paper throughout the book, but this also increases the weight of the volume. The bibliography is scarcely adequate for so comprehensive a book; it might have been better to have given to it the eight pages taken up by the list of equipment needed for a parasitological laboratory and the notes on the transport of diagnostic material. The book, nevertheless, strikes out a new line in the balance of its material, and anyone who has to deal with parasites encountered in European climates will find it a useful addition to his library. G. LAPAGE

EFFECTS OF SMOKING

Smoking

Its Influence on the Individual and Its Role in Social Medicine. By C. Van Proosdy. Pp. viii+319. (Amsterdam: Elsevier Publishing Company; London: D. Van Nostrand Company, Ltd., 1960.) 57*s*. 6*d*.

EVER since its introduction into Europe at the end of the fifteenth century, tobacco-smoking has met with periodic opposition from temporal and ecclesiastical authorities, and other self-appointed guardians of public health and morals. Much of the criticism of the use of tobacco has been ill-founded and based on ignorance or prejudice, but in recent years a considerable body of scientific evidence has accumulated which indicates that tobacco-smoking may have deleterious effects on the smoker's health. The relationship between cigarette-smoking and lung cancer is now well established, and there is evidence that cigarette-smoking may also be connected with some forms of heart disease and certain other diseases. Most smokers would probably argue that smoking has beneficial effects as well, and it obviously has very strong attractions. These appear to be associated with the nicotine content of the smoke, and psychological and social factors may also be important.

In his book, Dr. Van Proosdy has brought together a great deal of the available evidence relating to the medical, psychological and social aspects of smoking. This is a very wide field with an extensive literature, and the herculean nature of Dr. Van Proosdy's labours is indicated by the bibliography, which occupies forty pages of the book. The pleasures of smoking are not entirely disregarded, but the emphasis is on its dangers and drawbacks, and nearly half the book is devoted to a discussion of "The Damage done by Tobacco to the Human Organism". This reviews the epidemiological and clinical evidence and also, where available, the experimental evidence, relating to the influence of smoking on the development of cancer of the lung, larynx and mouth, on other diseases of the respiratory tract, on diseases of the heart and blood vessels and on the digestive organs, nervous system and sexual organs. Other chapters are concerned with the psychological and pharmacological aspects of smoking and the use of tobacco as a socio-psychological phenomenon. Techniques used for investigating the effects of smoking and possible methods of making smoking less harmful are also discussed.

The coverage appears to be fairly complete, at least in the field with which I myself am most familiar, and moderately well up to date with references to results obtained up to and during the year 1959. The large amount of information given in a relatively small book makes for succinctness; the book is to be recommended as a useful work of reference on the medical, psychological and social aspects of smoking. W. CARRUTHERS

FROM BUTTONS TO BULLION

Two Hundred Precious Metal Years

A History of the Sheffield Smelting Company Limited, 1760–1960. By Ronald E. Wilson. Pp. xxii+316+54 illustrations. (London: Ernest Benn, Ltd., 1960.) 63*s*. net.

IN these days of large corporations, it is rare to find a sizeable firm where a family has maintained a big interest for 200 years. The author of this book is a great-great-great-grandson of John Read, the founder of the Sheffield Smelting Co., which has been for a long time one of the most important precious-metal smelting firms in the country. He is thus in a unique position to survey the development of the firm which reflects, in some measure, the growth of Sheffield as a metallurgical centre. While this book will interest particularly those people familiar with the Sheffield scene, it provides the general reader with an accurate account of the early days of precious-metal smelting in Hallamshire. At first, the prosperity of the infant firm rested in the skill with which gold and silver were recovered from jewellers' waste or sweep. As the Sheffield plate trade developed in the latter half of the eighteenth century Read's business flourished and already by 1783 he was selling more than 50,400 ounces of silver a year in Sheffield. In the early days the business contacts of the firm already ranged from the Royal Mint to the scores of button makers in Birmingham and Sheffield.

By the end of the century, the firm was smelting waste from many parts of the country and from Europe. There is ample evidence that dealings in precious metals were never without risk, for much depended on the skill of the assayer and the accuracy of the sample, not to mention the reliability of the Company's agents, which varied from time to time. Add to this the habit of gold and silver prices to change unpredictably, and the occasional imposition of precious-metal taxes, and one has all the necessary ingredients for an exciting commercial life. Consequently, in the nineteenth century, the fortunes of the Company waxed and waned, and disaster threatened from time to time. However, it is a measure of the resilience of the family that the firm survived to become the leading precious-metal smelters in Britain towards the end of the nineteenth century. The part of the book dealing with the eighteenth and nineteenth centuries is likely to be of greatest interest because of the many sidelights both on the social life and industrial development of the times.

While the twentieth century led to continued expansion and broadening of activities, the actions of Governments could turn fair wind into foul, as when the U.S. Treasury ceased to support the silver market in 1935. The author is perhaps more reticent about progress after the Second World War, but it is clear that since then there has been great development and that the Sheffield Smelting Co. commands a very important position among the refiners and fabricators of precious metals.

This book is an interesting historical survey against an ever-changing social and economic background, with sufficient technological information for the reader to appreciate the development of this rather exotic industry from humble beginnings.

R. W. K. HONEYCOMBE

THE RETRIEVAL OF PUBLISHED INFORMATION

Retrieval Guide to Thermophysical Properties Research Literature

Vol. 1. Edited by Y. S. Touloukian. Book 1, Part A: Guide to Substance Classification and Numerical Codes. Book 1, Part B: Dictionary of Synonyms and Trade Names. Book 1, Part C: Directory of Substances. Pp. xii+1352. Book 2: Classified Search Index. Pp. vii+169. Book 3: Master Bibliography; Author Index. Pp. ix+372. (New York: McGraw-Hill Book Company, Inc.; London: McGraw-Hill Publishing Co., Ltd., 1960.) £46 10*s*. 0*d*.

THIS massive work constitutes the first fruits of one particular plan to master the enormous wealth of knowledge which is the outcome of scientific research. The general problem is well known. As laboratories are multiplied and scientists grow in numbers, any use of the information they obtain is liable to become increasingly patchy, because it is becoming increasingly difficult to locate every mention of a subject in published scientific literature. Often much of the information available has, in fact, not been used because its existence was not known. In quite a few cases there is wasteful duplication of effort in planning research to obtain scientific information that is already adequately available, for the purpose in view, in published literature.

The Thermo-Physical Properties Research Centre, Purdue University, has been working since 1957 to overcome the present limitations to the use of acquired knowledge in a strictly defined and fairly narrow field of thermophysical properties of matter. These aim to be those of importance primarily to engineers, and these three books of Volume 1 are the first publication from this Centre. They deal with 10,000 references concerning the thermal conductivity, specific heat, viscosity, emissivity and related optical properties, diffusion coefficient, and Prandtl number of a long list of materials. By the time Volume 3 is published, the aim is to include linear and cubical coefficients of thermal expansion surface tension, vapour pressure and *P.V.T.* data, a group of derived thermodynamic parameters, latent heats of fusion, critical and phase transformation constants, thermo-electric constants, and electrical conductivity.

One of the main goals of this *Retrieval Guide* is to reach the ultimate in comprehensiveness of coverage of information about these thermophysical properties, part of which may never be found by merely consulting the abstracting journals. To reach this goal, a high level of financial expenditure and intellectual effort has obviously been involved. An interesting feature is that unlike other collections of similar data, this group of books develops its own method of coding substances, which needs to be mastered carefully to use the interlocking method of coding properties and of the 10,000 literature references given in Book 3 of Volume 1.

It need scarcely be stated that effective retrieval systems are becoming of increasing importance for applied and even for pure science. Before deciding just how good the present system will prove to be, one would wish to proceed experimentally, and to expose one or two working librarians to the use of this volume by comparison with other methods of retrieving desired information from scientific literature.

Sampling suggests that the present publication is likely to prove most useful and to repay handsomely its initial cost for central libraries that deal with many inquiries. For smaller libraries, appropriate, if clumsier, books of tables of physical properties are likely to remain more acceptable for some time to come, even though they suffer from the grave defect of lack of comprehensiveness. The standing of the Research Centre is a warrant for the continuation of this series in further volumes. Their production is of high quality, and the series should prove itself to be increasingly indispensable as more volumes increase the range and dates of publication of the information covered. A. R. UBBELOHDE

DICTIONARY OF THE FUNGI

Ainsworth and Bisby's Dictionary of the Fungi

Fifth edition by Dr. G. C. Ainsworth. Pp. viii+547. (Kew, Surrey: Commonwealth Mycological Institute, 1961.) 30*s*.

AINSWORTH and Bisby's *Dictionary of the Fungi* has been regarded as a major work of scholarship since the publication of the first edition in 1943. It has earned for itself a place on the bookshelves of all interested in the study of fungi, and so the appearance of the fifth edition is warmly welcomed by mycologists and plant pathologists alike.

The new edition resembles closely the previous one. In the dictionary proper, which occupies 440 pages, the information is organized under headings arranged alphabetically on the lines of Willis's *Dictionary of the Flowering Plants and Ferns*. More than 5,000 corrections and alterations have been made to the text of the fourth edition, and much new material has been added. The inclusion of 500 more generic names than in the fourth edition ensures that most genera included in the *Index of Fungi* up to the end of 1960 are to be found in the *Dictionary*. The taxonomic position of each genus is indicated, together with the number of species in a genus and their distribution. Full attention is paid to synonyms. Excellent short accounts are provided of the chief families, orders and classes of Fungi and of bacteria and lichens. Definitions are given of many terms used in mycology and plant pathology, while the general articles on such subjects as collection and preservation of fungi, the genetics, nutrition, and phylogeny of fungi are valuable. Throughout the text there are numerous references to appropriate literature.

Following the alphabetical dictionary, 55 pages are devoted to listing the genera of fungi by classes, orders and sometimes by families. A revised key to the families of fungi by G. W. Martin occupies 21 pages. A new set of drawings illustrating fungal structure and terminology is provided.

The *Dictionary of Fungi* may be regarded as a mine of carefully compiled and well-presented information, being undoubtedly one of the landmarks of mycology. The new edition is good value at the price of thirty shillings. JOHN COLHOUN

The Major Achievements of Science

By A. E. E. McKenzie. Vol. 1. Pp. xvi+368+24 plates. 30*s*. net. Vol. 2: Selections from the Literature. Pp. xii+195. 17*s*. 6*d*. net. (Cambridge: At the University Press, 1960.)

IN the first of these two volumes the author attempts to describe, in twenty-four chapters, the progress made in various branches of science during the past four centuries. The first chapter introduces the subject with an outline of the development of science and technology from the Stone Age to the Renaissance; and four chapters, appropriately placed, give some account of the state of philosophy in each century from the seventeenth to the present, showing how the changes in outlook are related to advances in science. Of the remaining chapters, more than half are devoted to the physical sciences; chemistry occupies two chapters, geology one, and some aspects of biology, including medicine, five. Neither botany nor zoology is mentioned in the index. Chapter twenty-four is philosophical rather than historical.

This work is intended primarily as an introduction to science for students of the humanities, either in the sixth form at school, or at universities, and is an expression of the belief of its author that such an introduction is best effected by the historical approach. In furtherance of this object, the second volume consists largely of selected extracts from scientific literature that are historically significant, and it supplements, chapter by chapter, the text of volume 1. Again some philosophy is included. Most of the extracts are quite short, and have been chosen so that they offer no technical difficulties to the non-scientific reader. All are in English, many of the translations having been supplied by the author, Mr. A. E. E. McKenzie. As seems usual in modern works on the history of science, and is perhaps inevitable in the 'space age', there is a bias towards the physical rather than the biological aspects of science. With this limitation, and provided it is realized that, though covering a wide field, the work is not comprehensive, it can certainly be recommended as likely to serve its purpose. The book is well produced, and contains numerous attractive illustrations.

J. M. Eyles

Principles of Human Genetics

By Curt Stern. Second edition. Pp. x+753. (San Francisco and London: W. H. Freeman and Company, 1960.) 68*s*.

PRINCIPLES OF HUMAN GENETICS possesses all the attributes of the perfect text-book: a wealth of accurate, up-to-date, well-illustrated information evenly covering every aspect of the subject presented in a logical, intelligible and interesting way. The form of the first edition is retained in the second, but the book has been extensively rewritten to include the enormous advances that have been made during the past twelve years. These have particularly necessitated considerable revision of the sections dealing with linkage, sex determination, biochemical genetics, mutation and the role of selection, and completely new chapters are now included on polymorphism and the genetic hazards of radiation. True to its title the book is primarily concerned with principles and all the detail is presented in exemplifying these. Particularly meritorious is the way that Stern has managed to explain simply the basis to the mathematical and statistical procedures that are necessary in human genetics.

The recent studies of human populations have spectacularly elucidated many general genetic principles. This is, no doubt, why Stern's consideration of population genetics seems especially brilliant, but it would be unjust to single out any one topic for particular praise if by doing so it is implied that others are not as well treated as they could be. The only adverse criticism that might reasonably be made is that polygenic inheritance is not considered so comprehensively as the other topics, for no systematic attention is given to the statistical methods of analysing quantitative variation such as have been used by S. B. Holt in her rigorous study of dermatoglyphic patterns. However, in all, it is confidently predicted that *Principles of Human Genetics* will long be indispensable to those who study human biology, and many will be permanently indebted to the author of this magnificent book.

G. Ainsworth Harrison

Ovum Humanum

Growth, Maturation, Nourishment, Fertilization and Early Development. By Prof. Landrum B. Shettles. Pp. viii+78 (64 figures). (New York: Hafner Publishing Company, Inc., 1960.) 55*s*.

INTEREST in the development of the mammalian embryology dates from the dawn of medical science, and there was much speculation as to the origin of embryos. It was not, however, until 1827 that Von Baer discovered the mammalian ovum. More than one hundred years passed before a human ovum was recovered from the uterine tube by Allen *et al*. (1930). Once the mammalian ovum had been recognized the science of mammalian embryology was established on a sound basis.

The author of the present atlas is to be congratulated for giving us a pictorial presentation in sixty-four superb photomicrographs of the development, growth, maturation, ovulation, fertilization and cleavage of the human ovum.

In the captions of different illustrations the following topics, among many, are discussed and illustrated: the enzymatic action of the tubal mucosa on the corona radiata cells, the movements of the vitellus within the zona pellucida, the presence of two primary oocytes within a single follicle and its possible bearing in twinning, and the nourishment of the ovum by the cells of the corona radiata.

The book makes a valuable contribution to our knowledge of the development of the human ovum, and it can be recommended without reserve.

W. J. Hamilton

Mammals of the British Isles

By Philip Street. Pp. 185+32 plates. (London: Robert Hale, Ltd., 1961.) 21*s*. net.

THIS is an exceptionally lucidly written book, which will appeal to a wide public. It will provide easy and enjoyable reading for anyone wishing to improve his knowledge of the huge subject of mammals. It may, in some ways, be too straightforward for the specialist, yet even he will appreciate the interesting and well-organized treatment of fundamentals, and the fact that much of the information is based on recent research.

There are thirteen chapters to the book, an index, and fifty-six excellent photographs of mammals.

Although these are not colour plates, they are carefully produced pictures, all large enough to be detailed and revealing.

The first chapter is a general introduction to mammals, covering their evolution, and the distinctive features of all the Orders. The eight Orders found in Britain are dealt with in detail, including both wild species and alien species bred in captivity. From this chapter alone, an enormous amount can be learnt about mammals in general.

Chapters 2–12 deal, by groups, with the different species of mammals in Britain, including domestic animals, including domestic pets. Among a host of interesting information, there are accounts of origins, and of present-day efforts to preserve species that are gradually becoming extinct. The final chapter is on breeding animals for fur.

This would be an excellent book for a senior school library, or for any layman's shelf.

B. J. G. MAITLAND

A Biology of Crustacea

By Dr. J. Green. (Aspects of Zoology Series.) Pp. xv+180. (London: H. F. and G. Witherby, Ltd., 1961.) 30*s*. net.

IF the severe limitations of length are accepted, then this is an excellent book. It deals mainly with the biology of the intact animal, and it is said that the aim "is to provide a general background of biological knowledge for all who may encounter the Crustacea"; however, a background of biological knowledge is required to read this book. Although a good attempt is made to keep technicalities to a minimum, a fair number of terms are introduced without explanation.

Most chapters are over-condensed and, unfortunately, the first suffers most. This chapter endeavours to give a picture of the origin and radiation of the entire class, introducing, what, to the non-specialist, must be a bewildering array of names from sub-class to species. The account of radiation is confused by details of classification and it is suggested that the book would be much improved if the appendix (a systematic classification of the genera mentioned in the text) included a short list of the diagnostic characters of each group. Other chapters deal with feeding, growth, behaviour, blood, distribution etc., and the species used to exemplify the subjects are well chosen and from a wide geographical range. A considerable proportion of the subject-matter is taken from recent works. As might be expected, Dr. Green has drawn largely from studies of the Branchiopoda, but this certainly does not detract from the value of the book or invalidate the generalizations made. Typographical errors are few and errors of fact less.

J. A. ALLEN

Biochemistry of Steroids

By Erich Heftmann and Erich Mosettig. (Reinhold Organic Chemistry and Biochemistry Textbook Series.) Pp. xi+231. (New York: Reinhold Publishing Corporation; London: Chapman and Hall, Ltd., 1960.) 55*s*. net.

THE authors acknowledge the help of 28 well-known colleagues who reviewed part or all of the manuscript. It is not surprising, therefore, that this book is both well written and remarkably free from errors. Although the text occupies only 169 pages, the most important features of the biochemistry (with relevant chemical and physiological background where necessary) of cholesterol and related sterols, tetracyclic triterpenes, vitamin D, sapogenins, steroidal alkaloids, cardiac glycosides, bile acids, progesterone, corticosteroids, androgens and œstrogens are presented in a concise, readable style. At the end of the text, 774 references to recent books and reviews are appended; the headings and sub-headings to this bibliography simplify the search for more detailed accounts of selected topics. In addition, there is an extensive subject-index, which enables structural formulæ in the text to be found rapidly. Moreover, reference numbers in the bibliographic section are quoted in the subject index under the various entries, thereby facilitating a search of the literature. Honours students and those research workers who wish to acquire a working knowledge of an important and rapidly developing field with the minimum of reading will find this book very valuable. Unfortunately, the price suggests that their access to it is likely to be through the medium of a library.

D. T. ELMORE

Plant Viruses

By Dr. Kenneth M. Smith. (Methuen's Monographs on Biological Subjects.) Third edition. Pp. xii+209+8 plates. (London: Methuen and Co., Ltd.; New York: John Wiley and Sons, Inc., 1960.) 16*s*. 6*d*. net.

AT a time when virus research is so thriving, it is regrettable that plant viruses are so little studied in British universities. A book intended for "the student who has no previous knowledge of plant viruses" may encourage such study and is therefore very much to be welcomed, especially when its author is so experienced in plant-virus work as Dr. Kenneth Smith. But although the new edition of this little book contains more than twice as many pages as the old, one feels that it is still too small to do justice to the subject, which has grown immensely in recent years. No doubt, to limit the book's size, the author has left out many of the new lines of work which have developed in his "fourth period" in the history of virus study, starting in 1935. Thus, for example, the chemical nature of plant viruses is scarcely referred to, although the fact that all those yet sufficiently adequately studied have proved to be ribonucleoproteins is basic to any attempt to understand their stability, serological properties, multiplication, and methods of purification. Then again, the student, like myself, may not understand the techniques of X-ray crystallography in detail, but he should know more of what X-ray studies have contributed to knowledge of the structure of virus particles.

In the first part of the book are described symptomatology, modes of dissemination, electron microscopy and effects on physiology of host plants; all students of biology should read the chapter on the relation of plant viruses to their vectors. The chapters on practical methods in plant virology which form the second part cannot fail to benefit students. There is, however, little to indicate which of the methods described are of greatest practical use and which are the most reliable. Some, such as neutralization of infectivity, and anaphylactic shock, are unfashionable; some others, such as certain of the diagnostic colour tests, are unreliable.

In short, although there is plenty of good information for the student in this book, the spice of modern work might have been a little more liberally applied.

B. D. HARRISON

THE NATIONAL PHYSICAL LABORATORY

OPEN DAYS

MORE than 4,000 people visited the National Physical Laboratory during May 10–11, when items of the work of all the scientific divisions were on display. In addition to the usual number of invitations sent to industry, public and national boards and Government Departments, this year saw a greater number of visitors from the universities and colleges of advanced technology.

A new feature of the open days was the lectures given by Dr. H. Barrell on "Industrial Measurement Standards", and Dr. J. A. Pople on "Research in the Basic Physics Division", in the new lecture theatre of Glazebrook Hall.

The work of the Laboratory falls into three main categories, and examples from each of these were included in the programme for the two open days. The Laboratory has the national responsibility for establishing and maintaining the standards and units on which all measurements ultimately depend. Secondly, the Laboratory carries out basic research in the scientific disciplines covered by its nine divisions and publishes the results. Thirdly, the Laboratory assists industry by undertaking sponsored research, making tests and calibrations for fixed fees, and giving advice on problems that come within its scope.

It is impossible to describe, or even mention, all the items on display—more than 170 in all. But the three functions of the Laboratory can adequately be illustrated by a selection of exhibits covering each.

Standards

In October 1960 the eleventh General Conference of Weights and Measures adopted the metre defined in terms of the wave-length of the orange line of krypton-86. This marks the attainment of an objective towards which the National Physical Laboratory has been working since 1923. The use of metal bars as fundamental standards of length has been eliminated and another step taken towards the complete use of natural phenomena as fundamental standards in place of arbitrary material standards.

The Standards Division of the Laboratory celebrated this culmination of nearly 200 years of development of the metric unit of length by an exhibition of the "History of the Metre". Exhibits were shown beginning with the original Paris end-standard used by La Condamine in the measurement of the length of a degree at the equator and ending with the International Prototype Metre of 1875. The second group of exhibits showed some of Michelson's work, which estimated the possibility of measuring lengths of about 1 metre accurately in terms of wave-lengths of light emitted by a cadmium lamp.

The third group of exhibits showed developments since 1945 leading to the use of the orange line of krypton-86, the wave-length of which should be reproducible to an accuracy of one part in a hundred million. Recent work at the Laboratory, however, has been carried out with an accuracy approaching one in a thousand million.

The definition of the metre in terms of the orange line of krypton-86 maintains the previously accepted value of the wave-length of the cadmium red line in terms of the International Prototype Metre. (1 metre is 1 650 763·73 wave-lengths of the orange line of krypton-86.)

Industrial firms are always under pressure to meet increasing demands for greater precision in the manufacture of their products. They can be assisted in this by an increase in speed and accuracy in the calibration of standards used in engineering measurement. An exhibit was shown of recent work in the Standards Division which is effecting such improvements in the calibration of slip-gauges and angle standards. An automatic comparator is being developed in which slip-gauges are compared with reference standards by a pneumatic calliper. The calliper, automatically adjusted by a servo-system to accommodate different sizes, receives the gauges from a mechanical feed. Any known errors of the standard gauges are injected into the measuring system, so that the errors of the gauges being calibrated are shown directly on the machine. It is hoped that in the final model the results will be printed automatically. Visitors were informed that the machine should ultimately possess an accuracy of $\pm$ 1μ in. ($\pm$ 0·025 μm.), and will, therefore, be able to undertake work which is at present carried out by the much slower interference techniques.

A second exhibit showed the results of an investigation into some of the factors which influence the accuracy with which angle standards in the form of polygons can be calibrated. Photo-electric autocollimators were used for the experimental observations. An eight-sided steel polygon had been calibrated at three different establishments under normal industrial inspection-room conditions and it was found that some of the limitations imposed by commercially available equipment could be partially removed by improvements in its design and handling.

The Light Division staged a demonstration based on the illumination of prints of paintings and coloured fabrics, showing the latest developments in its work on colour-rendering or the ability of a light to give a correct presentation of colour values. This work has been made necessary by the general adoption of fluorescent and other discharge lamps, the light from which can depart considerably from the 'black-body' type of spectral distribution to which the eye is naturally adapted. A general theory of the tolerance of the eye to such departures, worked out in the Laboratory, has now been confirmed on a practical scale at the National Gallery and the Victoria and Albert Museum with the aid of numerous observers, and it will soon be possible to lay down rules for defining the effectiveness of colour rendering in lamp and lighting specifications.

Basic Research

The preliminary work on solids at extremely high pressures demonstrated last year by the Basic Physics Division has now been extended, and some very interesting results were shown. The apparatus demonstrated last year builds up pressures of the order of 100,000 atmospheres and creates, at the same time, working temperatures ranging from $-200°$ to $+2{,}000°$ C. The high pressures are

obtained by squeezing, with four anvils, the focus of a tetrahedral pressure cell of $\frac{3}{4}$-in. edge-length. High temperatures are obtained by heating electrically an inner cell containing the sample; low temperatures by circulating a cryogenic liquid around the anvils. Since the latter also serve as electrical contacts, the apparatus is suitable for making electrical conductivity measurements. These have been made on inter-metallic semi-conductor compounds. For example, indium antimonide has been found to change abruptly from the semi-conducting to the metallic state at 30,000 atmospheres and room temperature, a phenomenon probably due to the melting of the compound.

In addition to studying the behaviour of known materials, ultra-high pressure techniques may be used for the synthesis of new ones. The simultaneous application of high pressures and temperatures sometimes causes a re-arrangement of atoms in the sample under pressure. When normal conditions are restored, this arrangement remains 'frozen in', thus forming a new material. The Division has synthesized, in this way, coesite from quartz and diamond from graphite, the conditions for the latter transition being 80,000 atmospheres and 2,000° C. with nickel as a catalyst. Although diamond was first synthesized in the United States six years ago, this is the first time equipment with this capability has been set up in the United Kingdom.

The same Division displayed a technique for identifying new species of radicals in irradiated materials by utilizing spectron-spin resonance. Identification is by interpretation of the hyperfine coupling pattern. Emphasis is being laid on measurement of typical coupling constants in simple cases as a means of predicting the pattern to be expected for any particular chemical species. Work has been done on γ-irradiated amino-*iso*butyric acid; evidence has been obtained for CO_2^- in irradiated sodium formate.

The Metallurgy Division demonstrated how the use of the modern electron microscope has provided a means of studying the processes that occur during the ageing of iron. It had been thought for some time that the increase in strength of alloy steels above that of pure iron was due to fine dispersion of precipitate particles; but the nature of this strengthening process could not be investigated in its entirety until the advent of the electron microscope, since the particles were below the resolution of the optical microscope (about 1/100,000 in.). Particles less than 1/100 of this size can now be examined *in situ* in the metal. The specimen to be studied is thinned by electro-polishing until it is about 1/100,000 in. thick; it is then transparent to the electron illumination of the 100-kV. electron microscope. The internal structure can now be observed, both the precipitates and the lattice defects or dislocations being seen. It is the movement of these dislocations that causes plastic flow in metals, and where they can move easily, as in a pure metal, the metal is soft. It is thought that precipitate particles strengthen the metal by acting as obstacles to dislocations. The Division expects to be able to study the details of the interaction and to extend its investigations to commercial steels where the additional elements present can affect the precipitation processes.

The Ship Hydrodynamics Laboratory at Feltham displayed some new experiments to determine the effects of entrained water for typical arrangements of ship control surfaces. In the experiments a ship

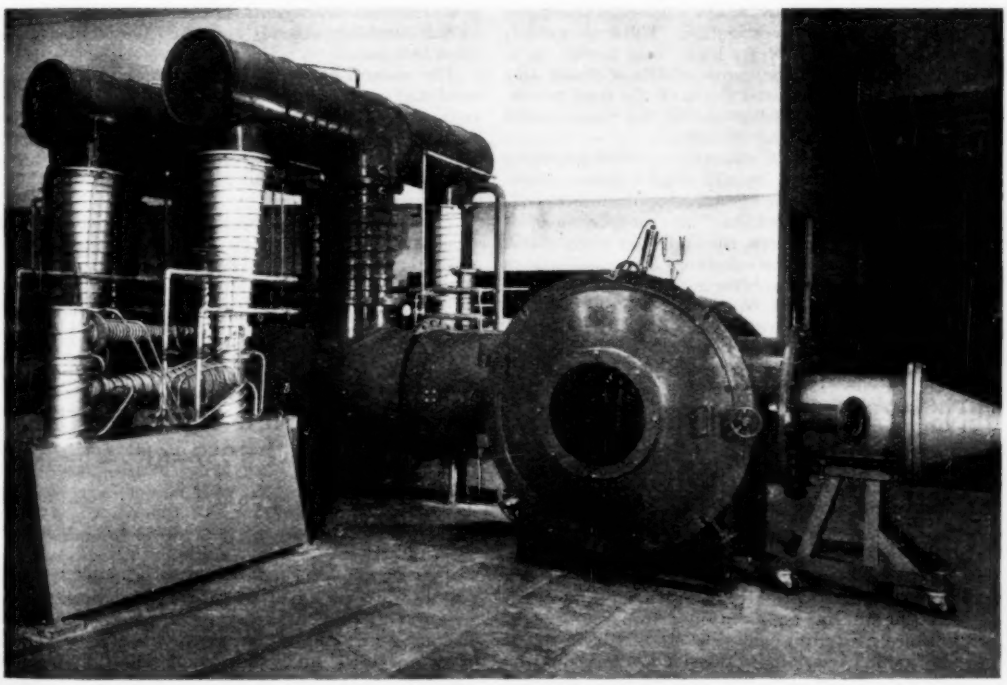

Fig. 1. New low-density tunnel of the Aerodynamics Division. Stagnation pressures, 0·0001–5 atmo.; Mach number range, 0–10
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rudder is represented by a vertical, rigid plate capable of rotating about a vertical axis and of lateral deflexion by bending this axis. This is then vibrated in either or both of its torsional and flexural modes and measurements made to distinguish between four sets of pairs of natural frequencies, with the assembly in air and in water, uncoupled and coupled. From these measurements, the Division will be able to calculate the hydrodynamic masses and moments of inertia for conditions of restraint corresponding to those in which ship control surfaces operate.

In the Aerodynamics Division, new hypersonic shock facilities were demonstrated, including a low-density wind-tunnel and plasma jet (Fig. 1), a 'hot-shot' wind-tunnel and a magnetically driven shock tube.

Some initial experiments were shown on the noise generated by air jets. The object of the work is to investigate the basic relationship between jet turbulence and noise.

Test and Research Work for Industry

The amount of work for industry carried out by the Aerodynamics Division is steadily growing. An interesting exhibit this year was that showing the aerodynamic stability investigation for London's new micro-wave tower, which has to be sufficiently high (500 ft.) to enable transmission and reception of micro-wave signals without interruption from other tall buildings. Periodic forces set up by vortex shedding tend to cause tall slender structures of circular section to oscillate in a bending motion when exposed to a steady wind. The exceptional dimensions of the new tower and its location make considerations of aerodynamic oscillations an important factor in its design. The design engineers therefore needed a wind-tunnel investigation. The scale-model, which was more than 8 ft. high, was tested in a 13 ft. × 9 ft. tunnel; its shape, elastic stiffness and mass distribution simulated those of the final tower, so that the oscillatory behaviour of the tower could be predicted from that of the model.

The sugar industry and manufacturers of products of biochemical interest should derive great satisfaction from the recording spectropolarimeter exhibited by the Light Division. The measurement of optical rotatory dispersion through the visible and ultra-violet regions of the spectrum has applications in the analysis of sugar mixtures and other optically active compounds. Up to the present time, this technique has not been appreciated fully by industry, because of the lack of an instrument which would give accurate results over a wide range of the spectrum. The new instrument demonstrated allows light transmission at the shorter wave-lengths; its range is 600–220 mμ, but this can be extended to 200 mμ by using prisms with better ultra-violet transmission. This facility would be of great value to those wishing to study biological compounds, since many of these are expected to have informative rotatory dispersion curves in the wave-length region of 200–260 mμ.

The merging of the Test House with the Applied Physics Division in 1958 made possible simplification and rationalization of thermometer testing. The display this year demonstrated improvements which will help to meet the increasing demands of industry for the testing of a greater quantity of instruments over wide temperature-ranges and with higher accuracy. Improvements include trunking of services, centralization of marking operations, mechanization of the production of ice-shavings and modernization of the testing baths to ensure uniformity of temperature and simultaneous testing of similar instruments. One of the chief advantages of the new layout is that it will link routine calibrations more closely with the final standard of reference, which is a platinum resistance thermometer as specified in the International Practical Scale of Temperature of 1948.

The Standards Division has provided the civil engineering industry with a new instrument which was demonstrated for the first time this year. It will be known as the 'NPL Microwave Mekometer' and rapidly measures long distances, with an accuracy of a few thousandths of a centimetre, by utilizing an elliptically polarized light-beam modulated at 9300 Mc./s. The distance of a reflector from the modulator is determined in terms of the wave-length of the polarization modulation. The device should be usable in full sunshine, since it is insensitive to background illumination.

The control of complicated industrial processes by trial-and-error methods is becoming accepted by industry. The Autonomics Division demonstrated analogue equipment for the study of adaptive control systems. Two items were shown, a simultaneous three-parameter controller and high-speed analogue computing equipment.

The Mathematics Division invited visitors to discuss with members of its staff the contents of the revised edition of its manual on modern computing methods which was on display, and also any computational problems of interest to them.

J. R. Illingworth

DENSITY OF THE UPPER ATMOSPHERE DETERMINED FROM SATELLITE ORBITS, AND ITS VARIATION DURING 1957–61

By D. G. KING-HELE and Mrs. D. M. C. WALKER
Royal Aircraft Establishment, Farnborough

In the past few years a consistent and satisfactory picture of atmospheric density at heights between 180 and 700 km. has been obtained from the rate of contraction of satellite orbits[1–5]. It has been found that the main variations in density are linked with: (1) solar activity and (2) changes between day and night. In this article (which is based on ref. 6, where fuller details are given) an improved method of calculating air density is applied to the orbits of 29 satellites to obtain a picture of the air density at heights of 180–700 km., which shows the general decrease in density as sunspot activity declined between 1957 and 1961, and the large day-to-night variation at heights greater than 400 km. The influence of latitude on density is also examined.

Method of Analysis

The air drag acting on a satellite rises to a maximum where it comes closest to the Earth, at the perigee, and is very small by comparison at heights more than about 100 km. above perigee. Therefore the integrated effect of air drag, which decelerates the satellite in the neighbourhood of perigee and hence causes the orbital period T to decrease, is a measure of the air density at some mean height within a band extending upwards for roughly 100 km. above perigee: and the best height at which to evaluate density is a little above that of perigee. If the air density ρ at heights above that of perigee is assumed to vary exponentially with height y above the Earth's surface, so that:

$$\rho = \rho_p \exp\left(-\frac{y - y_p}{H}\right) \quad (1)$$

where suffix p denotes perigee and H is taken as constant, the optimum height for evaluating air density is found[6] to be $\frac{1}{2}H$ above perigee, for orbits with eccentricity e between 0·02 and 0·2. The appropriate value of H varies with height and with time, and furthermore is not known exactly. Let H^* be the best estimate of H, subject to maximum errors of perhaps 30 per cent. Then it can be shown[6,7] that the air density ρ_A at a height $\frac{1}{4}H^*$ above perigee is given, with error less than 3 per cent if H^* differs from H by a factor of less than 1·5, by the equation:

$$\rho_A = -\frac{0{\cdot}158\,\dot{T}}{\delta}\sqrt{\frac{e}{aH^*}}\left\{1 - 2e + \frac{5}{2}e^2 - \frac{H^*}{8ae}\left(1 - 10e + \frac{7H^*}{16ae}\right) + \frac{\varepsilon}{e}\sin^2 i\cos 2\omega\right\} \quad (2)$$

if $0{\cdot}02 < e < 0{\cdot}2$. In this equation, $\dot{T}$ is the rate of change of the orbital period of the satellite, a is the semi-major axis of the orbit, ε is the ellipticity of the atmosphere (which may be taken as equal to the Earth's ellipticity, 0·00335), i is the inclination of the orbit to the equator and ω is the argument of perigee.

The quantity δ in equation (2) is given by:

$$\delta = \frac{FSC_D}{m} \quad (3)$$

where F is a factor allowing for rotation of the atmosphere (usually lying between 0·9 and 1), S is the mean cross-sectional area of the satellite perpendicular to its direction of motion, C_D is a drag coefficient, taken[6] as 2·2 for most satellites, and m is the mass of the satellite. In calculating S, it is assumed that the satellites have rotated about an axis of maximum moment of inertia, and S is taken as the mean of the cross-sectional areas for the two extreme modes of motion, which for a satellite with large length/diameter ratio are: (1) rotating like an aeroplane propeller, and (2) tumbling end-over-end. The estimated resulting error (s.d.) in δ is[6] between 5 and 10 per cent. These assumptions are the same as in ref. 1, except that the equation for ρ_A has been improved.

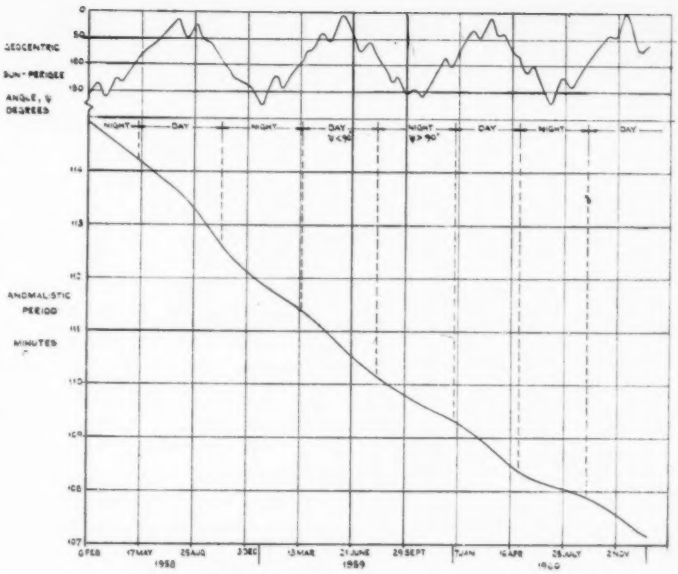

Fig. 1. Orbital period and geocentric Sun-perigee angle for *Explorer I*, 1958α

If $0 < e < 0{\cdot}02$, equation (2) becomes inaccurate, and it is found best to evaluate the density ρ_B at a height $\frac{1}{4}H^*$ above perigee height. ρ_B is given[6,7] by:

$$\rho_B = -\frac{\dot{T}}{3\pi a\delta}\cdot\frac{\exp(z + c\cos 2\omega - 0{\cdot}25)}{I_0 + 2eI_1 + cI_2\cos 2\omega + \frac{1}{4}c^2 I_0} \quad (4)$$

where $z = ae/H^*$, $c = \dfrac{\varepsilon a}{2H^*}\sin^2 i$, and I_n is the Bessel function of the first kind and imaginary argument, of order n and argument z. This equation is much less satisfactory than (2), having a maximum error not exceeding 9 per cent if H^* differs from H by a factor of less than 1·2. Fortunately, however, only 4 of the 92 values of density given in this article depend on equation (4).

Variations in Air Density

If the air density at a given height were constant from day to day, the orbital period of a satellite would decrease in a smooth and predictable manner. In practice, the rate of decrease is found to be erratic, and the irregular variations in air density thus revealed have proved to be linked with solar disturbances[9–14], the density being highest when the Sun is most active. In 1958, at sunspot maximum, the short-term fluctuations in density about its mean value from this cause sometimes amounted to ± 20 per cent at a height of 200 km. and ± 40 per cent at 700 km. This correlation also implies that there will be a long-term variation in density during the course of a sunspot cycle, a topic to be discussed in more detail later.

The variation in density between night and day is small at heights between 200 and 300 km., the density being about 10 per cent higher by day[3,10,15]. But above 300 km., this day-to-night variation is much

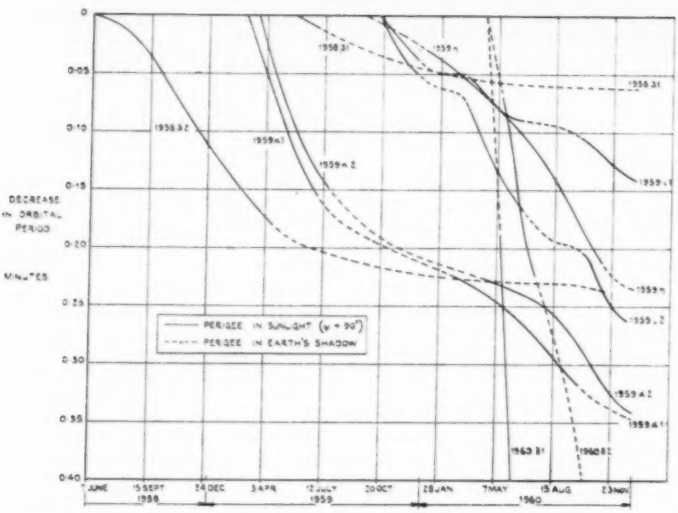

Fig. 2. Decrease in orbital period for satellites with perigee heights of 280–700 km.

greater, and is the dominant feature of the atmosphere. At a height of 600 km. the density is 5–10 times greater at its day-time maximum (attained in the afternoon) than at its night-time minimum (attained between midnight and dawn)[1–3] and consequently the temperature is several hundred degrees higher by day.

Changes in density between day and night have been clearly revealed by satellites, because their perigees move only slowly relative to the Sun: it takes at least two months and sometimes several years for perigee to accomplish a full day-to-night cycle. If therefore the orbital period of a long-lived satellite is plotted against time, the slope of the curve, $\dot{T}$, can be regarded as proportional to the air density ρ_A, since the quantities a, e, H^* and δ in equation (2) undergo little change. So day-to-night variations in density are at once apparent from the slope of the curve.

Fig. 1, which gives the orbital period of *Explorer* 1 (1958α, perigee height 350 km.), shows clearly that the slope is greatest by day and least by night. The quantity plotted at the top of Fig. 1 is the angle $\psi = SCP$ subtended at the Earth's centre C by the Sun S and the perigee point P: if ψ is greater than 90°, the point on the Earth's surface vertically beneath perigee is in darkness, and ψ equal to 90° is taken as forming a convenient boundary between 'day' and 'night'.

For orbits which are inclined to the equator at angles less than about 40°, the perigee moves eastward relative to the Sun, like a point on the Earth's surface, and experiences the normal progress from morning to evening conditions. *Explorer* 1, with inclination 33°, is in this category, and Fig. 1 thus shows that the slope of the period-versus-time curve (and hence the air density) increases only slowly during the morning, reaches a maximum some 2–3 hr. after noon, and then begins to decrease appreciably just before dusk, reaching a minimum between midnight and dawn.

The change in density between day and night has now been exhibited by nine other satellites with perigee heights between 280 km. and 700 km., and the decreases in their orbital periods are shown in Fig. 2: all the curves tend to be steeper by day (full lines) than by night (broken lines). Four of the curves, for *Explorer* 7 and its rocket (1959ι 1 and 2) and *Transit* 1*B* and rocket (1960γ 2 and 1), refer to orbits inclined at about 50° to the equator, for which the perigee moves westward relative to the Sun, going from evening to morning. The maximum slopes of these curves should therefore be attained soon after the perigee emerges into the evening sunlight. This expectation is confirmed by Fig. 2, most clearly for 1959ι2, while the slopes of the other curves, which refer to *Vanguards* 1, 2 and 3, increase much more slowly when the perigee enters the (morning) sunlight.

The decrease in density between 1958 and 1960 can also be recognized from Fig. 1. For if the density had been constant during these years, the 'average' slope of the curve in Fig. 1, with the oscillations smoothed out, would have tended to increase. In fact, it plainly decreases, thus reflecting a substantial decrease in density.

Influence of Latitude

The perigee latitude of a satellite usually varies widely, and since the value of $\dot{T}$ depends primarily on the density of the air near perigee, any strong and systematic variation with latitude would show itself in the period-versus-time curve. So far there has been no obvious sign of any such variation for the many satellites with inclinations less than 50°. For latitudes greater than 50°, evidence is so far available only for heights between 200 and 300 km., the best information being provided by *Sputnik* 3, for which the perigee moved slowly in the course of the life of the satellite from 50° N. to 65° S. and then back again to a point north of the equator. Fig. 3 shows the density of the air at a height of 235 km. as found from the orbit of Sputnik 3 by a method described in detail in ref. 6 : the error (s.d.) in the values is estimated as 7 per cent. The full line gives the calculated values: but, since some points refer to darkness and others to sunlight, a better comparison is obtained by correcting the values to conditions corresponding to the maximum day-time value of density, as shown

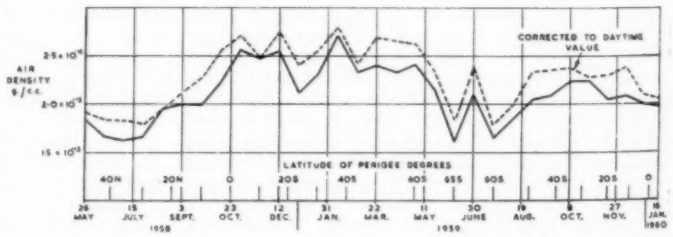

Fig. 3. Air density at height of 235 km., as given by *Sputnik* 3

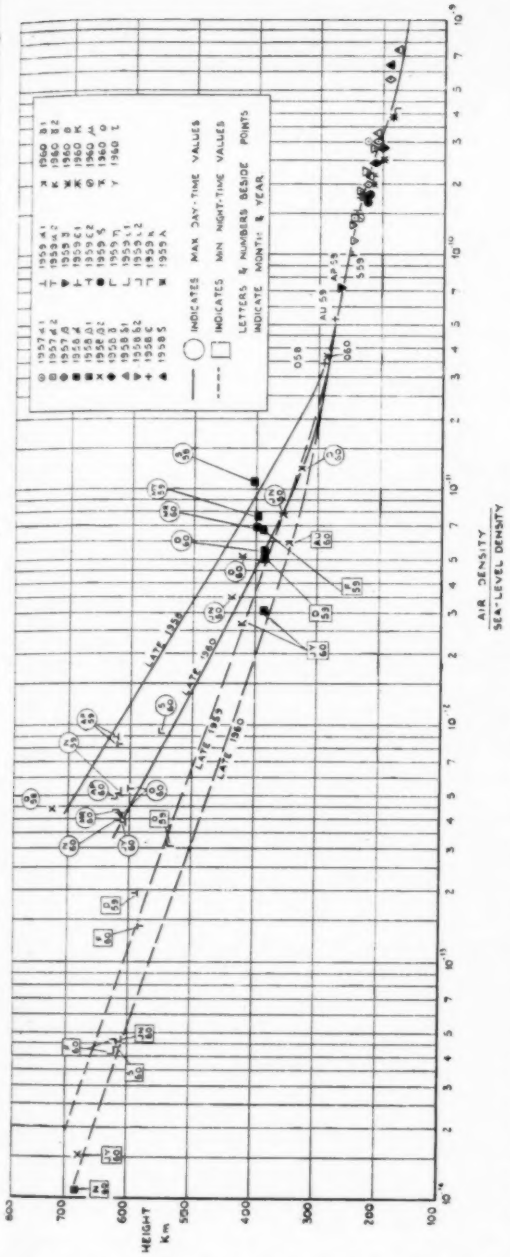

Fig. 4. Upper-atmosphere density obtained from the orbits of 29 satellites launched before January 1961

by the broken line. Because of the spread of drag about the perigee, the values of density can be regarded as referring to a band of latitude between 15° and 40° wide—the band being narrowest when perigee is at 65° S. and widest at the end when eccentricity is lowest and perigee near the equator. We can conclude from Fig. 3 that the day-time density at a height of 235 km. did not depart from its average value by a factor of more than 1·25, and most of this variation could well be ascribed to the influence of solar activity. Investigations of *Discoverer* satellites[6,15–17], with perigee latitudes up to 84°, have also revealed no sign of any significant variation of density with latitude.

Density of the Air during the Years 1957–61

In order to arrive at a general picture of the density of the upper air during the past four years, it is necessary to make due allowance for the well-established variations already discussed—the changes due to solar activity and between day and night. It is also advisable to utilize every possible satellite, so as to minimize the unknown individual bias associated with each.

Both these policies have been followed here. The curves of density-versus-height are divided according to date, that is, the particular year of the sunspot cycle, and a distinction is drawn between day- and night-time values. Also, a scrutiny was made of 66 satellites with the view of obtaining values of density: more than half these had to be rejected either because their weights and dimensions were unknown or because their orbits have not changed sufficiently. There remained 29 satellites, from which values of air density could be obtained, ranging from *Sputnik* 1 (1957α) to *Discoverer* 19 (1960τ).

The results are presented in Fig. 4, the density being expressed as a fraction of its sea-level value, taken as 0·00123 gm./c.c. The various satellites are distinguished by different symbols, and for heights above 300 km. each of the calculated points is labelled to show its date and whether it refers to day or night. The error (s.d.) in the majority of values of density, due to errors in δ and the orbital data, is estimated as about 15 per cent.

At heights less than 280 km. the points in Fig. 4 are closely packed, and a single curve has been drawn through them. Replotting of the points on a larger scale shows that they can be separated according to date, and that the density of the air at heights between 180 and 250 km. decreased by about 20 per cent between mid-1958 and mid-1960, so that the mean curve in Fig. 4 can be taken as referring to mid-1959. When, on the larger-scale diagram, curves appropriate to mid-1958 and mid-1960 were drawn, 27 of the 30 points at heights between 180 and 250 km. differed from the curve appropriate to their date by factors of less than 1·2. Thus the scatter among the points, which are quite independent, is rather less than would be expected from the estimated standard deviation of 15 per cent and, since the values refer to a wide variety of latitudes, Sun-perigee angles, seasons, etc., they confirm that there is little variation of density with any of these parameters at heights between 180 and 250 km.

At heights above 300 km. the curve in Fig. 4 divides into four parts: the two full lines indicate the maximum day-time density at the end of 1958 and of 1960; the two broken lines show the minimum night-time density at the end of 1959 and of 1960. Once again the points, although independent, fit into a consistent pattern. The curves indicate that the maximum day-time density decreased between late 1958 and late 1960 by a factor of about 2·0 at a height of 400 km. and about 2·7 at 600 km. The minimum night-time density decreased between late 1959 and late 1960 by a factor of about 1·8 at 400

Table 1. Values of Air Density and H given by Fig. 4

Height (km.)	Density (gm./c.c.)				H (km.)			
180	$6{\cdot}2 \times 10^{-13}$				35			
190	$4{\cdot}8 \times 10^{-13}$				46			
200	$3{\cdot}9 \times 10^{-13}$				53			
210	$3{\cdot}3 \times 10^{-13}$				58			
220	$2{\cdot}8 \times 10^{-13}$				55			
230	$2{\cdot}3 \times 10^{-13}$				51			
240	$1{\cdot}8 \times 10^{-13}$				39			
250	$1{\cdot}4 \times 10^{-13}$				32			
260	$9{\cdot}8 \times 10^{-14}$				32			
270	$7{\cdot}0 \times 10^{-14}$				34			
	Day		Night		Day		Night	
	Late 1958	Late 1960	Late 1959	Late 1960	Late 1958	Late 1960	Late 1959	Late 1960
300	$3{\cdot}6 \times 10^{-14}$	$2{\cdot}5 \times 10^{-14}$	$2{\cdot}8 \times 10^{-14}$	$2{\cdot}3 \times 10^{-14}$	70	46	46	39
350	$2{\cdot}0 \times 10^{-14}$	$1{\cdot}1 \times 10^{-14}$	$1{\cdot}1 \times 10^{-14}$	$6{\cdot}2 \times 10^{-15}$	94	74	57	47
400	$1{\cdot}2 \times 10^{-14}$	$5{\cdot}7 \times 10^{-15}$	$4{\cdot}5 \times 10^{-15}$	$2{\cdot}4 \times 10^{-15}$	96	81	58	52
450	$6{\cdot}9 \times 10^{-15}$	$3{\cdot}1 \times 10^{-15}$	$1{\cdot}9 \times 10^{-15}$	$9{\cdot}4 \times 10^{-16}$	97	84	↓	53
500	$4{\cdot}1 \times 10^{-15}$	$1{\cdot}7 \times 10^{-15}$	$8{\cdot}1 \times 10^{-16}$	$3{\cdot}9 \times 10^{-16}$	98	86	↓	↓
550	$2{\cdot}4 \times 10^{-15}$	$9{\cdot}6 \times 10^{-16}$	$3{\cdot}3 \times 10^{-16}$	$1{\cdot}6 \times 10^{-16}$	99	88	↓	↓
600	$1{\cdot}5 \times 10^{-15}$	$5{\cdot}5 \times 10^{-16}$	$1{\cdot}4 \times 10^{-16}$	$6{\cdot}6 \times 10^{-17}$	100	90	↓	↓
650	$9{\cdot}1 \times 10^{-16}$		$5{\cdot}7 \times 10^{-17}$	$2{\cdot}6 \times 10^{-17}$	101	92	↓	↓
700	$5{\cdot}5 \times 10^{-16}$		$2{\cdot}3 \times 10^{-17}$		102	94	↓	↓

km. and about 2·1 at 600 km. In late 1960 the maximum day-time density exceeded the minimum night-time density by a factor which increased from 1·1 at 300 km. height to 2·4 at 400 km., 4·3 at 500 km. and 8·3 at 600 km.

Numerical values of air density at the heights covered by Fig. 4 are listed in Table 1, together with values of the quantity H defined in equation (1). The individual values of H are obtained from the local slope of the density-versus-height curve and are therefore sensitive to the form of the curve in Fig. 4, with a possible maximum error of up to perhaps 30 per cent. But the average values of H over a height-range of, say, 100 km. should be much more accurate than this.

The air temperature can be determined[18] from H and the molecular weight M. Unfortunately, M is not known exactly at these heights. If, as is customary, M is assumed to fall from 25 at a height of 200 km. to 16 at a height of 500 km., and then to be constant up to 700 km., the following temperatures are obtained: at heights between 180 and 280 km. the average temperature is about 1,100° K.; between 400 and 600 km. it is, by day, 1,600° K. in 1958, decreasing to 1,450° K. in 1960, and by night about 900° K. in 1959–60. The error (s.d.) in these figures is probably about 15 per cent. At heights above 400 km. the temperature shows a tendency to be constant, in good accord with the theories of Nicolet[25].

The values of density given in Fig. 4 agree well with two independent determinations, from the expansion of a sodium cloud, and from manometers in *Sputnik* 3. The sodium vapour diffusion method[19] gave a density of $(6{\cdot}7 \pm 2) \times 10^{-15}$ gm./c.c. at a height of 430 km. in September 1958 at a time near sunrise. The corresponding value from Table 1, extrapolated and therefore less accurate than the tabulated values, is $(6 \pm 1{\cdot}5) \times 10^{-15}$ gm./c.c. The results[20] from the manometers in *Sputnik* 3, for example $8{\cdot}7 \times 10^{-14}$ gm./c.c. at 260 km., also agree well with Table 1.

Evidence on the density at heights up to 1,600 km. has now become available, from the orbit of *Echo* 1. At these heights, however, the validity of the method and the accuracy of the results are more questionable, particularly because of uncertainty about the drag coefficient and the effects of charged drag. For *Echo* 1, too, the effect of solar radiation pressure must be taken into account. The contribution of radiation pressure to $\dot{T}$, to be subtracted from $\dot{T}$, is given[21] by:

$$\dot{T}_s = 3Fh\left(\frac{T}{2\pi a}\right)^2$$

where F is the radiation force per unit mass acting on the satellite (to be taken negative) and h is the increase in the distance of the satellite from the Sun between entering and leaving the Earth's shadow. The values of air density obtained from *Echo* by various authors[22–24] are somewhat scattered: the values they derive, for maximum day-time conditions in 1960 at 1,500 km. height, lie between 10^{-18} and 5×10^{-18} gm./c.c.

We are grateful to the Smithsonian Astrophysical Observatory and the U.S. National Space Surveillance Control Center: many of the results given in this article are derived from the orbital data issued with their satellite predictions.

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LIMNOLOGICAL TRENDS IN LAKE KARIBA

By D. HARDING

Joint Fisheries Research Organization, Samfya Laboratory, Northern Rhodesia

ON December 2, 1958, the great dam across the Kariba Gorge was closed and Lake Kariba began to fill. The importance of hydrological study to the future Lake had been appreciated before this event, however, and water analyses, etc., had been collected from the Zambezi River in the future dam basin since 1956 [1]. This work was done by the Joint Fisheries Research Organization from its base at Samfya, and it was even at that time hoped that at least one permanent scientist would be appointed to do research work at Kariba on a full-time basis. Up to the present, however, this has not been possible owing to the lack of the necessary funds, though the importance of hydrobiological research at Kariba has been stressed by various authors [2]. Sufficient data have nevertheless been obtained by periodic visits to the Lake to form a good general picture of the trends from the time of closure, though unfortunate gaps in our records have occurred from time to time. But further work is essential to make the most of Kariba, not only as a producer of hydro-electric power but also as potentially one of the most important fishing industry centres in Central Africa. Similar schemes, also, are in various stages of progress in other parts of Africa, and Lake Kariba, which at high water will cover 1,087,000 acres and be about 175 miles long by 50 miles wide, offers a unique opportunity for research in a variety of fields, which will be of value to all concerned with the lakes so formed by these projects.

The observations from this fifth largest body of water in Africa, and largest man-made lake in the world, which are presented here, were mainly collected at a station about a mile from the dam site in Kariba Gorge, between February and December 1960. The depth of water here at a lake-level of 1,500 ft. above mean sea-level (as against an eventual height of 1,580 ft. above mean sea-level) was a little more than 70 metres. Other records have been taken elsewhere on the Lake and are mentioned briefly. As the Lake filled in 1959, the few records of temperature distribution with depth which were taken at the wall of the dam indicated that no permanent stratification had taken place by the time high-water mark for the year had been reached in July. The next available record is for November of that year, and this showed a distinct thermocline at approximately 10 m. In February 1960, this thermocline was still well established at 12 m. [3] and records of oxygen distribution in depth at several widely separated stations on the Lake indicated that only the epilimnion above the discontinuity layer contained oxygen (Fig. 1). Below this level there was no trace of oxygen even in shallow sheltered areas, although in the middle of the shallow western basin the Lake was 50 per cent saturated with oxygen down to the bottom at 15 m. As the year progressed into the cold season the epilimnion cooled and the thermocline moved gradually deeper under the stress of winds at the surface of the Lake. On May 26, 1960, the thermocline was still well established at 30 m.; the water above was homothermal at 25·3° C., and dissolved oxygen was again limited to this layer. Stratification broke down on July 6, 1960, and the Lake 'overturned', the water being homothermal at 23·4° C. Unfortunately, no oxygen records were taken at this period, but on June 28 the epilimnion was approximately 35 per cent saturated down to 36 m., when the temperature difference between surface and bottom water was less than 1° C. and the thermocline just detectable at 40 m. Shortly after the period of overturn, on August 23, when the Lake was homothermal at 22·2° C., records of dissolved oxygen showed a gradual decline from 65 per cent saturation at 4 m. to 35 per cent saturation at 51 m., and a gradual increase to 45 per cent saturation at 70 m. Similar conditions prevailed at the end of September. Warming began in late August, and by November 1, 1960, temperature conditions were similar to those of November 1959, a thermocline being well established at 12·5 m., and on December 2, 1960, dissolved oxygen was again confined to the epilimnion, which was approximately 17·5 m. deep.

Isotherms for the nine months between February and November illustrate the regime of temperature variation in Lake Kariba during 1960 (Fig. 2). A study of these isotherms indicates the presence of a layer of colder water in the deeper part of the Lake in October, which may be attributed to a source other than by direct mixing with the surface water in this area. The station is sited over the old Zambezi River channel in the gorge and is the deepest part of the Lake. Thus one would expect colder water from as far afield as the inflowing Zambezi to maintain its identity in the submerged Zambezi River channel and eventually accumulate in the deepest part of the Lake in Kariba Gorge. This

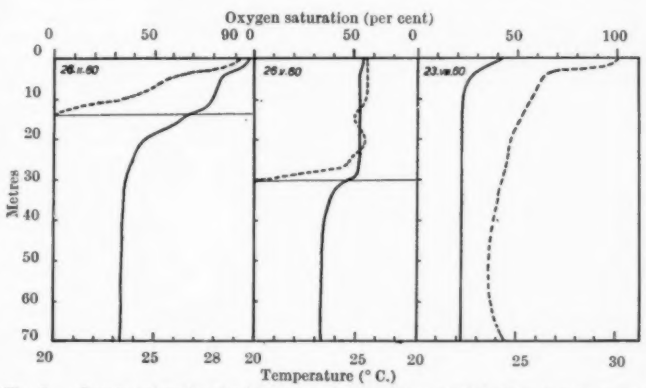

Fig. 1. Oxygen saturation (broken line) and temperature at three dates in Lake Kariba

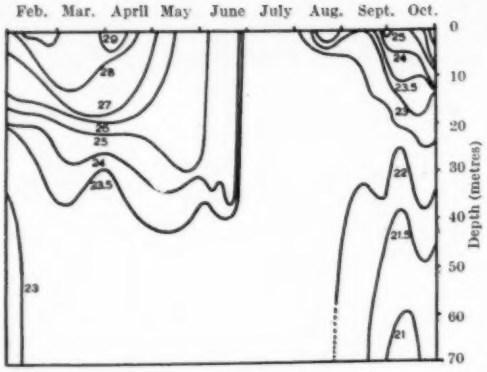

Fig. 2. Isotherms in Lake Kariba, 1960

water also differed from the overlying water mass in its higher dissolved oxygen content (Fig. 1), which tends to support this theory.

No mention of Kariba would be complete without reference to the water fern *Salvinia auriculata*, which has covered between 150 and 200 square miles of the surface of the Lake during the past two years. Apart from the mechanical effects of choking backwaters and river estuaries, and blocking access routes to cleared fishing pitches, harbour sites, and indeed the main Zambezi River channel itself, a number of side effects, such as that on the dissolved oxygen content of the Lake water, have been observed during recent work in the affected areas. Long-established patches of the fern, in sheltered backwaters and river estuaries, have prevented exchange taking place with the atmosphere, resulting in deoxygenation of the water lying beneath the floating mat of closely packed plants. The more mobile patches of *Salvinia* have no such lasting effects on the water beneath, and in the main Zambezi inflow and inflowing river estuaries, oxygen values are relatively high beneath the mat (Table 1). The effects of water movements in the open Lake also tend to maintain a circulation beneath patches of *Salvinia* bordering on the open water. Thus a transect beneath a stable patch, which has been immobilized among semi-submerged trees in the Songwe estuary, showed an oxygen gradient decreasing from a saturation value of 71 per cent in the open Lake to 37 per cent approximately two miles into the weed, then increasing once again to 69 per cent in an area of open water a mile farther on near the shore.

Table 1. Dissolved Oxygen in Lake Kariba, 1960

	Percentage saturated*	
	February	April
River Zambezi at Devil's Gorge	140	—
River Zambezi near River Masumo confluence beneath *Salvinia* mat	74	78
River Songwe estuary beneath *Salvinia* mat	62	6
River Songwe estuary 8 m. beneath *Salvinia* mat	69	2
Open lake near River Chezia estuary	93	79
Open lake at edge of *Salvinia* mat	93	66
Beneath marginal *Salvinia* mat	67	39
Beneath marginal *Salvinia* mat at 5 m.	43	24

* Oxygen saturation values corrected for an altitude of 1,500 ft. above sea-level.

Table 2. Chemical Analyses of Surface Water from Lake Kariba*

	October samples		
	1958	1959	1960
Total solids dried at 180° C.	103	74	69
Loss of weight on ignition	25	15	9
Specific conductivity at 20° C. ($\times 10^4$)	1·21	1·00	0·93
Dissolved salines deduced from conductivity	69	58	52
Total hardness as $CaCO_3$	49	45	42
Chloride	3	2	1
Sulphate	3	2·5	None
Nitrate nitrogen	None	None	None
Nitrite nitrogen	None	None	None
Ammoniacal nitrogen	0·06	0·05	0·06
Albuminoid nitrogen	0·26	0·21	0·13
Bicarbonate	72	66	62
Calcium	14	14	13
Magnesium	3·6	3·0	2·0
Total iron	None	None	None
Sodium	8	7	5
Potassium	None	None	None
Silica (SiO_2)	10	8	11
*p*H	7·3	7·2	7·2
Oxygen-absorbed test, 4 hr. at 27° C.	1·2	1·8	1·5
Main combinations:			
Calcium carbonate†	35	33	32
Magnesium carbonate†	12	10	8
Sodium carbonate†	11	10	10
Sodium sulphate	4	4	0
Sodium chloride	5	3	2
Total of above	67	60	52
Plus silica (SiO_2)	10	8	11‡

* These analyses were carried out by the Government Analyst in Salisbury, Southern Rhodesia.
† Present as bicarbonate.
‡ Results in milligrams per litre.

The implications of reduced oxygen tensions either by thermal stratification or by mats of *Salvinia auriculata* are obviously important to the fishery in Lake Kariba. On one hand, the process of stagnation of water below the thermocline, followed by an overturn, may be of general benefit to the Lake fishery, in so far as the nutrient salts released to the hypolimnion under anaerobic conditions as a result of the decomposition of drowned vegetation are made available for the growth of certain elements of the plankton and ultimately to the fish. On the other hand, the extensive oxygen-poor hypolimnion will prevent the development of the profundal fish fauna, and *seiche* movements could result in killing juveniles and breeding fish, should deep water devoid of oxygen reach the surface near the margins of the Lake. The extensive *Salvinia* mats may also exclude fish from their breeding grounds or, by deoxygenating water close in shore, result in deaths of juvenile fish. Furthermore, the position of *Salvinia auriculata* is such that salts leached from the surrounding land during a succession of annual inundations will be immediately available to the plant and this may well be the reason for the 'explosive' growth of the fern.

Conditions in Lake Kariba during 1960 simulate the cycle of events which occur in the surface layers of the deep tropical lakes Nyasa and Tanganyika. Stratification occurs during the hot season, accompanied by a depletion of oxygen below the thermocline. This is followed by a breakdown of stratification during the cold season, which results in a complete overturn in Lake Kariba; but because of their great depths and in some cases a second thermocline, only the upper levels of Lakes Nyasa[4] and Tanganyika[5] are affected, the abyssal water remains isolated and reduced, and there is never a complete overturn. Furthermore, even when Lake Kariba is full, almost a third of its total volume will be replaced each year from the inflowing rivers. Thus large quantities of nutrients will be available for biological production

from two sources, the inflows and the bottom deposits, and for this reason the Lake may be extremely productive. Chemical analyses (Table 2) show there has been an overall decrease in the total concentration of dissolved solids since 1958, which may be accounted for by remarkable plankton blooms, *Salvinia auriculata* and the expansion of the fish population during the past two years.

Limnological research at Kariba during 1961 will include such specific studies on the abundance of phytoplankton, the hydrology and chemistry, the ecology and abundance of fishes and the autecology and control of *Salvinia auriculata*, and will continue throughout the period of filling, which is expected to be completed in 1963/64, until the Lake enters its permanent phase of development.

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OBITUARIES

Prof. J. H. Andrew

THE death on May 5 of Prof. John Harold Andrew, at the age of seventy-four at Ruthin Castle Hospital in North Wales, was no surprise to his friends; he had been in ill health for a number of years, and Mrs. Andrew, to whom he was devoted, had died in January. Andrew retired from the chair of metallurgy in the University of Sheffield in September 1950, having been advised medically to take this step two years before the end of his statutory period of office; he was made emeritus professor. For reasons of health and because they loved the country, he and Mrs. Andrew retired to Llandudno.

A native of Ashton-under-Lyne, he was educated at Bickering House School, Birkdale, and the University of Manchester, where he graduated in chemistry in 1907 and was awarded the degree of D.Sc. in 1915. In 1910 he was appointed a Research Fellow and demonstrator at Manchester, where he was a Dalton Scholar. After six years as head of the Metallurgical Research Department of Sir W. G. Armstrong Whitworth and Co., Ltd., he was, in 1920, appointed professor of metallurgy at the Royal Technical College, Glasgow, succeeding the late Dr. C. H. Desch. With an established reputation in the field of metallurgy, he again succeeded Desch as professor of metallurgy and dean of the Faculty of Metallurgy at Sheffield in 1932.

Andrew was an original member of the Institute of Metals, was elected a member of the Iron and Steel Institute in 1911 and was a founder Fellow of the Institution of Metallurgists. He was a prominent member of the Heterogeneity of Steel Ingots, Alloy Steel Research and Steel Castings Research Committees of the Iron and Steel Institute, all which have now been incorporated in the committees of the British Iron and Steel Research Association.

During his thirty years as a professor at Glasgow and at Sheffield he published a very large volume of research covering extensive fields of interest. As a director of research, his fertility of mind, his unorthodox points of view and his continuing encouragement were a source of inspiration to many postgraduate students. The number of joint papers he published bears testimony to his great sense of loyalty and co-operation.

Andrew was not only well known for his research work. His administrative ability and great talent for organization brought about a striking development of the Departments of Metallurgy at Glasgow and at Sheffield. In recognition of his work in metallurgy, he was awarded in 1949 the Bessemer Gold Medal of the Iron and Steel Institute, an honour which he richly deserved. Shortly after his retirement in 1950, a group of Sheffield steel firms established the J. H. Andrew Senior Research Fellowship tenable in his old department, where a laboratory is also named after him.

To his staff, from the most senior to the humblest laboratory assistant, he was a friend who, with his sympathetic understanding and lively sense of humour, made difficulties seem trivial.

Prof. and Mrs. Andrew leave friends in Glasgow and Sheffield in all walks of life who will not forget their unfailing kindness, courtesy and generous hospitality. It was the hope that some of the happiness they had given should have been theirs in retirement; unfortunately, their retirement was sadly marred by continued ill-health. W. R. MADDOCKS

Dr. H. Horn af Rantzien

ON September 15, 1960, Dr. Henning Horn af Rantzien was killed at Montmorency, France, by a stone which slipped while, in the company of his wife and a French friend and colleague, he was examining a quarry for fossil plant remains.

Henning Horn graduated at Stockholm and, from 1945 on, held research positions in the Botany and Palæobotany Departments at the Riksmuseum, Stockholm, and later in the Geology Department of the University of Stockholm, where he taught historical geology. During 1948–51, he was an editor of *Svensk Botanisk Tidskrift*. At the time of his death, he was pursuing a long-term research programme on the taxonomy and phylogeny of the Charophyta, a work to which he had devoted most of his time during the past ten years and which had carried him, at the age of thirty-eight, to a leading position in the field of micropalæontology.

Those who knew him only as a highly specialized palæobotanist may be surprised to learn that a great part of his early writings were on recent vascular plants. He paid particular attention to the taxonomy and distribution of *Phleum*, *Pleurospermum*, and the West African and Latin American species of *Mayaca*, *Najas*, and *Triglochin*. Simultaneously, he revised the American *Nitellae* and thoroughly reviewed the literature available on all American Charophyta. In 1951, following extensive field-work, he published an ecological–geographical study of aquatic and amphibious plant associations (including bryophytes

and charophytes) of the shallow lakes and temporary pools of the peculiar 'alvar' limestone barrens of the island of Öland, in the Baltic. It was in the course of this work that his interest in the charophytes became definitively established.

This broad approach did not shatter his conviction that only on a firm morphological basis can useful work be done on plant systematics and distribution. Therefore, when extending his attention to the fossil Charophyta, he ventured on a detailed description of the fructifications of modern, Cenozoic and Mesozoic forms. In doing this work he developed his own techniques for preserving and examining these minute objects. The newly recognized value of such fructifications as layer markers over a wide range of geological strata, from Upper Silurian to late Tertiary, lends special importance to the results he achieved. It is sad to reflect that this voluminous pioneer work was but the first step on a road which he was not to complete.

Privately, Henning Horn had a gift for finding pleasure in all manifestations of living Nature and in the most diverse fields of learning. He took particular interest in history and archæology, and in the life-history of land vertebrates (he was an expert herpetologist). A shyness which he never quite overcame usually caused him to avoid gatherings of more than a few people, a fact much to be regretted, for his versatile mind, coupled with a great sincerity as soon as basic facts were under discussion, made him an impressive and uncommonly stimulating partner in discussions with friends within and outside his own scientific domains. He is survived by his wife and five children.

C. Edelstam

NEWS and VIEWS

Royal Institution Fullerian Professorship of Physiology : Prof. R. J. Harrison

The Managers of the Royal Institution have elected Prof. R. J. Harrison to be Fullerian professor of physiology, Royal Institution, in succession to Prof. J. Z. Young. Prof. Harrison is University professor of anatomy in the London Hospital Medical College, a post he has held since 1954. He was educated at Oundle School and Gonville and Caius College, Cambridge, and received his medical training at St. Bartholomew's Hospital Medical College. He has held appointments in the University of Glasgow and Charing Cross Hospital Medical School, and is the author of *Anatomical Terms* and *Man the Peculiar Animal*, in addition to numerous papers on embryology and anatomy. He is especially known for his research on the physiology of diving seals. The Fullerian professorship of physiology was endowed in 1834 by the gift of John Fuller, an early generous benefactor of the Royal Institution. P. M. Roget was the first holder of this office. Richard Owen, Thomas Huxley and Sir Charles Sherrington are among the other previous Fullerian professors. The appointment is normally for a period of three years.

Research on the Effects of Radioactivity in the Sea : Prof. Ilmo Hela

Prof. Ilmo Hela, at present director of the Institute of Marine Research, Finland, has been appointed chief scientist in charge of the programme of research on the effects of radioactivity in the sea, to be conducted in Monaco under a trilateral agreement between the International Atomic Energy Agency, the Government of the Principality and the Institute of Oceanography. Prof. Hela is expected to take up his new appointment at the beginning of October. Prof. Hela was educated in the Universities of Helsinki, Königsberg and Hamburg. He received his Ph.D. in oceanography from the University of Helsinki in 1945; earlier he had studied physics and meteorology as well. After several years research work at the Institute of Marine Research in Finland, he joined the Marine Laboratory of the University of Miami as associate professor of oceanography and meteorology in 1951. In 1955 he returned to the Institute of Marine Research as director and professor. The programme of research in Monaco, which Prof. Hela will direct, has three major objectives. First, it will be aimed at acquiring knowledge about the movement of water and marine organisms and the deposition of organic and inorganic matter. Secondly, there will be a special study of the distribution in marine organisms of radioactive materials already existing or that may be introduced into various locations. Finally, investigations will be carried out on the effects of radioactive materials at various concentration-levels on the marine ecology. The research will be conducted at the Oceanographic Museum in Monaco, but the laboratories and working facilities of the scientific centre of the Government of the Principality, including a wide variety of electronic and monitoring equipment, will also be available.

Mathematics at Leeds : Prof. J. W. Craggs

Dr. J. W. Craggs, reader in applied mathematics in the Newcastle division of the University of Durham, has been appointed to the newly created chair of mathematics in the University of Leeds. Dr. Craggs graduated with first class honours in mathematics at the University of Manchester and afterwards obtained the degree of doctor of philosophy both at Manchester and Cambridge. At Cambridge he held a senior studentship awarded by the Commissioners for the Royal Exhibition of 1851. Dr. Craggs has had teaching appointments at the Military College of Science, at the University of Manchester, University College, Dundee, and at King's College, Newcastle upon Tyne, University of Durham. He has been at Newcastle since 1951 and was later appointed to a senior lectureship and then to a readership. Dr. Craggs's research interests have ranged over a wide field. His first work was concerned with potential and diffusion problems and with three-dimensional elastostatics. He then moved on to compressible fluid-dynamics and more recently has made important contributions to problems of wave-propagation in elastic and plastic bodies.

The Leopoldina German Academy of Sciences : Elections

The Deutsche Akademie der Naturforscher Leopoldina, Halle, recently elected the following new members in the sections indicated: *Physics*, Prof. Helmut Hönl (Freiburg-Br.). *Chemistry*, Prof. T. R. Seshadri (Delhi). *Biology*, Sir Ronald A. Fisher (Cambridge). *Agriculture*, Prof. Walter Fuchs (Göt-

tingen). *Anatomy*, Prof. Joachim Hermann Scharf (Halle). *Physiology*, Sir John C. Eccles (Canberra). *Pathology*, Prof. A. von Albertini (Zürich); Prof. Ernst Letterer (Tübingen). *Biochemistry*, Prof. Marcel Florkin (Liège); Prof. Otto Westphal (Freiburg-Br.); Prof. Edgar Lederer (Paris). *Hygiene*, Prof. Walter Liese (Berlin); Prof. Josef Tomesik (Basle). *Stomatology*, Prof. Richard Trauner (Graz).

Scientific Societies and the Rating and Valuation Bill

At the Committee stage of the Rating and Valuation Bill in the House of Lords on June 22, Lord Brabazon of Tara, speaking as President of the Royal Institution, moved an amendment removing from the Bill the repeal of the Scientific Societies Act, 1843. Emphasizing the national importance of the scientific work which the Royal Institution had done during the past 160 years, and was still doing, he said that under the Bill the Royal Institution, which received no State funds of any kind, would be called on to find a further £3,000 a year, and scientific societies altogether would have to find £128,000 a year. Lord Brabazon indicated his willingness to withdraw the amendment if the Treasury could give any assurance that such a sum would be made available for the purpose to the Joint Committee of the Royal Society and the British Academy, and did in fact do so after hearing Lord Hailsham's reply and on the understanding that his point about the Royal Institution's Laboratory would be borne in mind on Clause 9 on the Report stage. Lord Hailsham fully agreed as to the national importance of the scientific work of the Royal Institution, mentioning also its library, but did not agree either that the Royal Institution should be treated separately or that the anomalies of the Scientific Societies Act should be perpetuated. Confirming all that he had said earlier as to the value of the work of the scientific societies and the needs of the societies, Lord Hailsham maintained that the correct course was to examine sympathetically any hardship resulting from the Act in the context of their needs.

The International Council of Scientific Unions, 1961

The Year Book of the International Council of Scientific Unions, 1961, includes, besides the calendar of arrangements for 1961, the usual details of the membership of the Executive Board, the National and Scientific Members of the Council, of the Special Committees, Joint Commission and Executive Bodies of the Permanent Services of the International Council (Pp. 110. The Hague: International Council of Scientific Unions, Paleis Noordeinde, 1961). There are also the statutes and bye-laws of the Union rules for Special Committees and their charter. The organization of the Commissions of the several Unions, the relations of the International Council with the specialized agencies of the United Nations, the alphabetical list of members of the Bureau, Executive Board, Special Committees, Joint Commission, and other organs of the Council and of officers of the Scientific Unions add further to the value of a most useful and handy work of reference, which well displays the manifold ways in which scientific work is interlocked at the international level.

Scientific Research in the U.S.S.R.

At a recent conference in Moscow attended by two thousand Soviet scientists, impressive figures were given of the Soviet research effort. Government spending on science in 1950 was 874 million (old) roubles, but by 1960 this had increased to 3,260 millions and during 1961 it is expected to reach 3,800 millions. Last year there were more than 354,000 scientific workers, about 11,000 of whom had a doctor's degree and 100,000 had a master's degree. There were 3,800 scientific establishments, including 1,500 research institutes. Academician Keldysh, president of the U.S.S.R. Academy of Sciences, outlined a new national research plan. He proposed that such a plan should be in three parts: the first to deal with economic problems at the experimental stage; the second for long-range projects aimed at solving already known problems; and the third to investigate the laws of Nature to open up new fields of progress. He described as "Task No. 1" the total electrification of the entire country. In that connexion the problem of finding new sources of power was of vital importance.

Oil Consumption in the United Kingdom during 1960

Following precedent, the Petroleum Information Bureau (on behalf of the U.K. Petroleum Industry Advisory Committee) has published recently statistics relative to oil consumption in Great Britain for 1960. (*U.K. Petroleum Industry Statistics relating to Consumption and Refinery Production 1959 and 1960*. Pp. 8. London: Petroleum Information Bureau, 1961.) The trend continues upwards, there being a 16·9 per cent increase in the total consumption of oil compared with 1959, reaching more than 42·5 million tons (excluding bunkers for ships engaged in foreign trade). Of the major oil products, fuel oil registered the greatest increase by more than 25 per cent over 1959, reaching a total of 17,437,805 tons. Gas/diesel oil deliveries were up by 13 per cent. Motor spirit consumption accounted for 7,625,184 tons, an increase of 7 per cent over the 1959 total. Diesel-engined road vehicle fuel (*DERV*) consumption rose by 13·5 per cent, a pointer to the growing demand for this commodity. As might also be expected, there was a remarkable uprise in the amount of chemical feedstock delivered, totalling 1,562,060 tons compared with 1,067,265 tons in 1959, representing a 46·4 per cent increment. This record reflects the magnitude of present-day commercial activities concerned with 'petro-chemicals' as distinct from traditional petroleum products, and at the present state of evolution of this now vital branch of the industry a commensurate increase in these 'by-products' may be anticipated confidently for 1961. There was in the year under review an increase in demand for bitumen, much of which was due to extension of the road-construction programme in so far as asphalt surfacing is concerned.

Shortage of Scientific Teachers in the United Kingdom

In answer to a question in the House of Commons on June 22, Sir David Eccles gave the number of vacancies at March 31, 1960, for teachers of mathematics in the technical colleges as 64 in an authorized establishment of 1,464; for physics the corresponding figures are 61 and 893, and for chemistry, 55 and 994. About 1,500 full-time women teachers employed in maintained grammar schools had degrees which included mathematics. Surveys made last year by the Incorporated Association of Headmasters and the

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Association of Headmistresses showed that about 100 grammar, including maintained, independent and direct grant, schools had unfilled vacancies for teachers of mathematics. In addition, about 370 schools had posts which were not filled with teachers having satisfactory qualifications. About 225 graduates with at least a pass-level degree standard in mathematics out of nearly 3,100 students were at present taking the teacher's diploma in university departments of education. Of the 62 general training colleges for women, only 45 had full-time lecturers in mathematics and 14 had part-time lecturers. Five such colleges and 8 mixed colleges provided specialist courses in chemistry and physics; 6 colleges and 11 mixed colleges provided main courses with full-time lecturers in chemistry or physics; 51 women's colleges and 21 mixed colleges had no full-time lecturers in chemistry or physics.

Replying to further questions in the House of Commons on June 29 about the supply of teachers of science and mathematics, the Minister of Education, Sir David Eccles, said he believed that the universities now realized the importance of the problem. He had asked local education authorities last November to take the initiative in providing local courses for serving teachers in those subjects, such as mathematics, in which there was a shortage of well-qualified teachers. Institutes of education had been asked to collaborate closely and he would ask university extra-mural departments to consider if they could help. Industry was already lending many teachers of science and mathematics to the technical colleges. Research into methods of teaching mathematics in primary schools was being undertaken by the National Foundation for Educational Research, and close attention to this was also being given by the inspectorate, training colleges and university departments of education, teachers and local education authorities. Many local short courses for teachers being arranged resulted in setting up of study groups for informal research. Much experimental work was being carried out, and Sir David agreed to take a special interest in seeing that the results of these experiments were diffused throughout the teaching service.

Trade in European Birds for Caging

In the autumn of 1960 information was received from the chairman of the Belgian National Section of the International Council for Bird Preservation, Monsieur E. Kesteloot, of the considerable export of live wild birds to England, particularly goldfinches and serins. As outlined in the British Section's annual report for 1960 (Pp. 38+4 plates. London: International Council for Bird Preservation, British Section, c/o British Museum (Natural History), 1961. 3*s*.), these birds are exported both direct and also through Belgium from other countries. Though in Great Britain goldfinches and other birds may not be sold alive unless they are close-ringed specimens bred in captivity, there is reason to believe that this law is being evaded. In 1956–57 the Royal Society for the Protection of Birds successfully prosecuted three different dealers who had imported hawfinches and other birds from Belgium and forced close-rings on their legs. The British Section has set up a sub-committee, consisting of Guy Mountfort (chairman), Miss P. Barclay-Smith, P. J. Conder, R. H. Spencer and R. S. R. Fitter as honorary secretary, to make recommendations which will control this unfortunate practice. A request has also been made for the subject to be discussed by the Conference of the European Section of the International Council for Bird Preservation which is being held in Norway.

Science in Space

The publication of a report by the National Academy of Sciences—National Research Council, entitled *Science in Space* (Washington, D.C.: National Academy of Sciences—National Research Council, 1961), which consists of nine chapters (each published as a separate volume), has now been completed with the issue of the four remaining chapters (Chapters 1, 6, 8, 9). The report is divided as follows: Chapter 1, Dimensions and Problems: a general review; Chapter 2, The Nature of Gravitation; Chapter 3, The Earth; Chapter 4, The Moon; Chapter 5, The Planets; Chapter 6, The Sun; Chapter 7, Physics of Fields and Energetic Particles in Space; Chapter 8, Galactic and Extragalactic Astronomy, and Chapter 9, The Biological Sciences and Space Research. The aim of this report is to interest scientists working in the fields covered by the nine chapters of the report in the possibilities offered by space research. As such the report is of value since the major problems in each of the above fields are concisely discussed with reference to the relevance of research in space for their solution. In this connexion each chapter contains a short factual section relating these problems with our present knowledge of the respective field of study and a short bibliography of some of the more important recent references. However, since the background is surveyed so concisely and the outstanding problems are presented with such clarity and with the minimum of technicalities, the report could well serve as an introduction to some of the problems confronting astronomy and physics at the present time. In particular it could influence the young research worker in the choice of a fruitful field study. Each chapter (costing one dollar) can be obtained from the Publications Office of the National Academy of Sciences, 2101 Constitution Avenue N.W., Washington 25, D.C., but it is hoped to produce the report, revised and expanded, in book form published by the McGraw-Hill Book Co., Inc., New York.

British Museum (Natural History): Recent Acquisitions

Among the more important recent acquisitions of the British Museum (Natural History) is the unique collection of British Microlepidoptera belonging to the late Mr. L. T. Ford. This collection, of upwards of 40,000 specimens, is stated to be the best of its kind in existence, its unique merit being the high proportion of material which was reared by Mr. Ford himself. Most species are represented by long series such as are indispensable for modern systematic research, and all are provided with full and accurate data. It forms the basis of Ford's published work.

Museum of Applied Science of Victoria

The report of activities of the Museum of Applied Science of Victoria for the year ended June 30, 1960, records progress in the construction of a new wing adjoining the Museum and also in the establishment of the Radiocarbon Dating Laboratory. (Pp. 23. Melbourne: Museum of Applied Science of Victoria, 1961.) The persistent difficulties with the latter work have now been overcome and the complicated equipment has functioned satisfactorily and age determinations of check specimens have been made. Several new major displays, including those illus-

trative of economic minerals and metals, economic botany, and the deterioration of timbers, have been set up. It should be noted that as from January 23 the name of this Museum has been changed to the Institute of Applied Science of Victoria.

Swimming Ability of Reptiles

SYSTEMATIC experiments have been carried out by R. R. Tercafs to test the swimming ability of non-aquatic reptiles (Bulletins de la Société Royale de Zoologie d'Anvers. No. 20. Pp. 19 (6 planches). Anvers: Société Royale de Zoologie d'Anvers, 1961). Among snakes, the following species have been studied: *Vipera verus*, *Boa canina*, *Thamnophis sauritus*, *Coronella austriaca*, *Chlorosoma schottii*, *Vipera ammodites* and *Eryx johnii*. The first two species cannot co-ordinate their movements, nor keep their heads out of water; the remaining species can swim very well, by movements similar to those they make on the ground. Some lizards (*Trachydosaurus rugosus*, *Tarentol mauritanica*, *Chamaeleo dilepis* and *Anguis fragilis*) have the same behaviour: they go forward in water by walking (thus using the same movements as on the ground). On the other hand, other species (*Lacerta viridis*, *Tiliqua scinoides*, *Lacerta muralis*, *Crocodilurus lacertinus*, *Iguana iguana* and *Tropidurus torquatus*) change their usual movements and swim in the same way as aquatic Caudata: they keep their members close to the body and go forward by waving with body and tail. The adaptation to swimming seems to be more ethological than morphological; the inability to swim of some reptiles might be explained by the lack of nervous co-ordination in response to stimuli in water; in the same way integration of these stimuli could explain the adaptation to swimming of some reptiles. Two adaptive stages exist, co-ordinated movements like the movements on the ground and swimming movements, identical with those of aquatic Caudata.

Reactions of Salt Solutions and Calcium Chloride on Mortar and Concrete

THE December issue of the *Australian Journal of Science*, 2, No. 4, December 1960) contains two significant communications to concrete specialists; one by J. A. Roberts and H. E. Vivian on "The Widening of Expansion Cracks in Mortar by Salt Solutions", the other by M. Lawrence and H. E. Vivian on "The Action of Calcium Chloride on Mortar and Concrete". In the former paper a new mechanism is described by which pre-existing cracks in mortar are enlarged and expanded by salt solutions, for example calcium nitrate and sodium silicate. Action of salt solutions tends to accelerate disintegration of concrete by opening of fine cracks therein, especially where the concrete is exposed to weathering conditions, for example roads. A conclusion is reached that concrete road-surfacings may be damaged by application of such salts as calcium chloride for the purpose of removing ice. It may be that certain aggregates may be similarly affected by salt reaction. In the second paper cited, it is shown that severe reaction, causing disruption of mortar and concrete, occurs when strong calcium chloride solution comes into contact with them. The disruption is similar to that caused by sulphate attack, but in this case it is considered to be due to formation of chloroaluminate, derived from the 'susceptible' compound in cement paste, tricalcium aluminate hydrate. It is anticipated that control of severe sulphate or chloride attack may develop from further work by employing "some method of rapidly modifying the susceptible hydration products of tricalcium aluminate and tetra-calcium aluminoferrite to ensure that no reactable material is available after the cement sets".

U.S. National Science Foundation: Office of Antarctic Programs

AN Office of Antarctic Programs has been established within the National Science Foundation. This new Office replaces the Antarctic Research Program, formerly a part of the Foundation's Office of Special International Programs. Dr. Thomas O. Jones has been appointed head of the new Office. Dr. Albert P. Crary has been appointed chief scientist and will also serve as science adviser on Antarctica to the director, Dr. Alan T. Waterman. The Office of Antarctica Programs is responsible for the United States Antarctic Research Program, which is the name used in the field to include all U.S. scientific work carried out in Antarctica. Scientists from colleges, universities and Government agencies, working under sponsorship of the National Science Foundation, carry out research in biology, geodesy and cartography, geology, glaciology, meteorology, oceanography, upper atmosphere physics and seismology.

U.K. Courses in Nuclear Energy, 1961–63

A CATALOGUE entitled *Nuclear Studies* has been compiled by the Science Department of the British Council. This lists courses in pure and applied sciences concerning the use and development of nuclear energy which may be undertaken in the United Kingdom. These courses are full-time, and are generally of one week or more duration. The information provided by the catalogue includes notes on each of the courses and the institution at which it takes place; also the fee and the qualifications required for participation. Further information can be obtained from the Science Department, The British Council, 65 Davies Street, London, W.1.

Eleanor Roosevelt International Cancer Fellowships

THE International Union against Cancer, by means of funds made available by the Eleanor Roosevelt Cancer Foundation, will award annually fellowships for research on cancer. These are senior post-doctoral awards designed for the support of persons who, as full-time members of the staff of universities, teaching hospitals, research laboratories or other institutions, have demonstrated interest and ability or promise as independent investigators in the field of research on basic cancer, and who wish to broaden their experience by a period of study in another country. The duration of the fellowships ordinarily will be one year, but this period may be extended or shortened in special circumstances. The stipend will be based on the current salary of the applicant and the salary of persons of comparable qualifications in the place where the applicant expects to study. Application forms can be obtained from the International Union against Cancer, P.O. Box 400, Geneva 2.

Nutrition and Metabolic Defects

THE Nutrition Society is organizing a symposium on "Nutrition and Metabolic Defects", to be held at the Laboratory of Physiology, Oxford, on July 15. The programme will include the following subjects: historical aspects of inborn errors of metabolism, by Dr. H. M. Sinclair (Oxford); clinical aspects of

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inborn metabolic defects, by Dr. Philip Evans (London); nutrition in relation to phenylketonuria, by Dr. L. I. Woolf (Oxford); the metabolism of copper and Wilson's disease, by Prof. J. N. Cumings (London); chronic magnesium deficiency, by Dr. Paul Fourman (Cardiff); the possible role of dietary factors in the ætiology and pathogenesis of sprue, cœliac disease and idiopathic steatorrhœa, by Prof. A. C. Frazer (Birmingham). Further information can be obtained from Miss D. F. Hollingsworth, c/o Ministry of Agriculture, Fisheries and Food, Great Westminster House, Horseferry Road, London, S.W.1.

Royal Statistical Society

At the annual general meeting of the Royal Statistical Society, Prof. M. G. Kendall was re-elected president for the ensuing year. Dr. B. Benjamin, Dr. P. Armitage and Mr. B. P. Emmett were re-elected honorary secretaries, and Mr. C. T. Saunders honorary treasurer. Prof. David R. Cox, professor of statistics in the University of London (Birkbeck College), was awarded the Society's Guy Medal in silver.

Royal Society and Nuffield Foundation Commonwealth Bursaries

Awards under the Royal Society and Nuffield Foundation Commonwealth Bursaries Scheme have been made as follows:

Mr. L. G. Campbell, of the Imperial College of Tropical Agriculture, Trinidad, to enable him to study aspects of drainage and irrigation in Ontario and Alberta during July and August, 1961.

Dr. Rosalie F. Ewer, research associate, Rhodes University, to assist her to study fossil material of the early archosaur reptile *Euparkeria* at University College, London, for four months from September 1961.

Dr. K. A. Kermack, lecturer in zoology, University College, London, to enable him to examine in museums in South Africa specimens of reptiles which were the ancestors of the Mammalia and to make collections from the beds of Uppermost Triassic age in South Africa, from October 1961 for five months.

Dr. D. F. McMichael, curator of molluscs, the Australian Museum, Sydney, to assist him to spend about four months from October 1961 at the British Museum (Natural History) studying the collections of molluscs with special reference to the type specimens of Australian and New Guinea land and freshwater molluscs housed there.

Dr. W. Macnae, lecturer in zoology, University of the Witwatersrand, to enable him to spend 6–8 months from December 1961 based on the University of Queensland and studying the ecology of the *Cymodocea* fields and mangrove swamps on shores within the Great Barrier Reef and in northern Australia.

Dr. J. N. Phillips, of the Commonwealth Scientific and Industrial Research Organization (Australia) Division of Plant Industry, Canberra, to enable him to extend his physico-chemical studies on plant and animal pigments at Imperial College of Science and Technology, London, for about eleven months from December 1961.

Dr. Muriel L. Tomlinson, university demonstrator in organic chemistry, Oxford, to enable her to study polydentate compounds at the University of Sydney for about three months early in 1962.

Prof. D. Walker, professor of physics, Victoria University of Wellington, to enable him to make a study of experimental research work at present of interest to workers with low-energy Van de Graaff accelerators (or similar machines), and to study the working of the National Institute for Nuclear Science in Britain, for eight months from June 1962.

Dr. H. B. S. Womersley, reader in botany, University of Adelaide, to assist him with maintenance costs at Vancouver for three months from April 1962 while making ecological comparisons of the intertidal biology (especially marine algae) of southern Australia and the western North American coast.

Announcements

Sir Howard Florey, president of the Royal Society and professor of pathology in the University of Oxford, and Dr. Owen H. Wangensteen, chairman of the Department of Surgery, University of Minnesota, and formerly president of the American College of Surgeons, have been elected Honorary Fellows of the Royal College of Surgeons of England.

Mr. G. C. Tootill (Royal Aircraft Establishment, Farnborough) has been appointed chairman of a special committee to consider international standards work on information technology by the International Federation of Information Processing Societies.

An international conference on elementary particles will be held during September 14–20 at Aix-en-Provence, France. Further information can be obtained from the Scientific Conference Secretariat (E. W. D. Steel), CERN, Geneva, 23, Switzerland.

The Technical Section of the British Paper and Board Makers' Association is organizing a conference on "The Formation and Structure of Paper", to be held at the Dyson Perrins Laboratory, Oxford, during September 25–29. Further information can be obtained from the Technical Section, British Paper and Board Makers' Association, St. Winifred's, Welcomes Road, Kenley, Surrey.

Under the auspices of a committee appointed by the Conchological Society of Great Britain and Ireland and the Malacological Society of London, the first European Malacological Congress is to be held in London during September 17–21, 1962. The object of the Congress is to discuss all aspects of European Malacology and the formation of a European Malacological Union. The Congress is open to all interested in Malacology, and further information can be obtained from the honorary secretary, Rev. H. E. J. Biggs, 19 Siward Road, Bromley, Kent.

The Institute of Physics and the Physical Society and the British Radio Spectroscopy Group are jointly holding a two-day conference on "Radio Spectroscopy of Solids" in the Department of Physics, University College of North Wales, Bangor, during September 21–22. The Institute and Society's Guthrie Lecture will be presented during the conference by Dr. D. Shoenberg, who will speak on "The de Haas–van Alphen Effect and the Electronic Structure of Metals". Further information can be obtained from the Administration Assistant, The Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

Errata. In the review of Prof. Fraenkel's *Abstract Set Theory* (*Nature*, June 17, p. 1047) the publisher was given as Elsevier Publishing Company, of Amsterdam. This should have read North-Holland Publishing Company, Amsterdam.

In the report on the Arthur D. Little Research Institute (*Nature*, 190, 1068; 1961), five lines from the end, for 'acid' read 'non-acid'.

SHORTAGE OF MATHEMATICS TEACHERS IN BRITAIN

A QUESTION was asked by Lord Boothby in the House of Lords as to whether, having regard to the essential part played by school mathematics in the training of scientists and technologists, and in view of the present shortage of more than 3,000 mathematical teachers in British schools and the fact that replacements were not matching retirements, the Government now proposes to take action to avert the incipient disintegration of technological training in Britain.

In replying on June 7, Lord Hailsham said that the Government has already taken action to meet the serious shortage of mathematics teachers in the schools, which it recognizes to be a matter of serious concern. He did not accept Lord Boothby's conclusion. The grave shortage of mathematics graduates able and willing to teach has been made known to the university authorities, and consultations are taking place with them about how the problem could best be tackled. The Minister of Education has also taken measures to improve the supply of non-graduate teachers of this subject.

Lord Boothby then referred to action taken by the National Science Foundation in the United States, of which full details had been given to Lord Hailsham, and to the recommendations of the Mathematics Conference at Southampton last April; but Lord Hailsham insisted that the core of the problem is the provision of graduates and that this is the province of the universities. As regards non-graduate teachers, the provision of a three-year as distinct from a two-year course has enabled the Minister of Education to raise the standard in teacher-training colleges. Lord Hailsham promised to note Lord Boothby's suggestion about better co-ordination between the Ministry of Education, his own Ministry, the University Grants Committee and the universities. He believes that there are difficulties in Baroness Summerskill's suggestion of a special financial premium for those teaching science and mathematics, and in reply to Lord Alexander of Hillsborough said that the anxiety of parents in relation to the present teaching of mathematics is not well founded. One factor in the shortage is the whole range of new opportunities now open to mathematics graduates. What is really wanted is an increase in the total output of graduates, and he is not satisfied we have as yet exhausted the potentialities of mathematical ability. He said that personally he is not satisfied that the methods of teaching mathematics could not be improved, so as to attract some who had hitherto been repelled. He thought that the shortage of mathematics teachers is likely to become, and is already, very much worse than the general shortage of teachers.

In a written answer in the House of Commons on June 13, the Minister of Education said that as a result of his Department's request to training colleges to give greater prominence to mathematics, the annual number of students reading mathematics as a main subject has risen from 770 in 1955–56 to 1,650 in 1960–61. The three-year course should greatly help to raise academic standards, and the colleges should then be sending forward to the primary and secondary schools more and better teachers of mathematics. He expected the numbers to rise further with the expansion of the colleges. The colleges are also providing one-year courses for substantial numbers of serving teachers, and various other shorter courses are also available for serving teachers and members of mathematics staffs in the training colleges. Advanced courses in the technical colleges leading to a degree or diploma in technology are also being expanded. The main problem is the shortage of mathematics graduates, particularly those able and willing to teach. Mathematicians in schools and universities had been actively discussing the question and canvassing possible solutions. He said he has made sure that the gravity of the shortage and the need for action are known to the university authorities. His Department has been working for some months with the Teachers' Advisory Council, and the universities have been approached through the Vice-Chancellors' Committee. It is proposed to hold a conference at an early date under university auspices to examine the evidence collected and consider possible action, and Sir David promised to bear in mind the suggestion of a White Paper.

It might be noted here that in the House of Commons on June 8, in replying to a question about difficulties anticipated in recruiting scientific staff for the universities expansion programme, Sir Edward Boyle said that the increase in recent years of the number of Department of Scientific and Industrial Research postgraduate training grants—from 979 in 1958 to 1,259 in October 1960—would contribute materially to the supply of potential teachers. Besides considering representations about a further salary revision, the University Grants Committee is at present giving particular attention to the provision of adequate facilities for university staff, both for teaching and research (see also p. 103 of this issue).

THE WELSH COLLEGE OF ADVANCED TECHNOLOGY

By Dr. A. HARVEY
Principal of the College

AN additional building for the Welsh College of Advanced Technology, situated in Cathays Park, Cardiff, was formally opened by H.R.H. The Duke of Edinburgh on June 2, the ceremony forming part of the Welsh programme for Commonwealth Technical Training Week. The building re-houses the three Departments of Chemistry and Biology, Navigation and Pharmacy. It also provides much improved amenities for the College as a whole, including a refectory, great hall, senior common room and a self-contained Students' Union suite.

The architect was Sir Percy Thomas, who was also (in conjunction with Mr. Ivor Bishop) responsible for the main building of the College, which was com-

[*Photo: Hylton Warner*

Fig. 1. Extension of the Welsh College of Advanced Technology

pleted in 1916. The college buildings are about 100 yards apart, being separated by the Temple of Peace, for which Sir Percy was also the architect (1938). The main contractors were Messrs. J. Gerrard and Sons, Ltd., of Swinton, the cost of the building being £626,724, and that of the furniture £66,600. The equipment, most of which is now installed, is costing approximately £150,000.

The structure is steel-framed and the exterior faced with Portland stone, so as to harmonize with the other buildings in the Civic Centre. The floors were made from pre-cast concrete units and have a variety of finishes, depending on the use to which a particular room is put. It is worth noting that the use of battleship lino in the corridors has largely eliminated the noise problem often encountered. All the usual services, including compressed air, steam and vacuum, are available, housed either in ducts or above the ceilings, and the layout of the services is such that any non-laboratory type of room can, when necessary, be converted into a laboratory. A goods lift is available for heavy or bulky supplies, and there are small electrically operated hoists in the science departments. Thus bulk supplies are received on the ground-floor and distributed from there, as required, to store rooms on the upper floors.

The Department of Chemistry and Biology occupies the three floors comprising the east side of the building, and its accommodation includes ten main laboratories with ancillary balance and preparation rooms. There are four research laboratories and two lecture rooms. The specialized provisions worth noting are for polymer chemistry and technology (two laboratories, a flame-proof room and workshop for the preparation and testing of plastics and rubber products) and for work with radioisotopes (laboratory, counting room, dispensary and an underground store).

Before the War this Department was well known as a research centre and during the late 1930's eight students obtained the Ph.D. degree, four the M.Sc. degree. Now, after a rather lengthy break, research is developing once more. Work in progress at present includes a series of studies in polymer chemistry and technology dealing with rubber vulcanization, rubber mastication, molecular shapes of polypropylene glycols and viscoelastic properties of epoxide resins. Other work includes a theoretical study of counter-current extraction, electrolytic dissociation, uptake of fall-out products by lower plants and studies of vitamin C deficiency.

Because of the importance attached to the education and training of industrial chemists, special attention has been paid to instrumental analysis. A wide range of instruments, including ultra-violet and infra-red recording spectrophotometers, is housed in an instrument room, equally available for instruction and research. In addition to this a laboratory has been equipped for the ultimate analysis of organic compounds.

The Welsh School of Pharmacy occupies the whole of the three floors forming the south side of the building. It thus has available some 21,000 sq. ft. of accommodation which includes sixteen teaching laboratories, eight research laboratories, together with nineteen other rooms (lecture rooms, stores, preparation rooms, etc.). The largest lecture theatre will seat 150 and has been so sited that it can be used, in

conjunction with an adjacent committee room and cloakroom, as a self-contained suite available for professional bodies and societies.

This Department is responsible for the education and training of pharmacists who may enter hospitals, industry or retail work. The College is affiliated, in respect of this work, to the University of Wales, so that students can take either the B.Pharm.(Wales) or the pharmaceutical chemist qualifying course. The Department has been designed for an annual intake of 64 students for a three-year course, but this could be increased to 96 without much difficulty.

The Department is an active research centre and is recognized by the University as a place for research for M.Pharm., while application has been made for recognition for Ph.D. There have now been seven M.Pharm. awards to members of the College.

Research at present in progress in the Department includes: physico-chemical studies on phosphatides; kinetics of the bromination of phenolic aldehydes; synthetic studies on rotenoids; synthesis of 2 : 2 dimethylchromenes which may have physiological activity; synthesis of apomorphine derivatives; synthesis of sulphones as antibacterials; properties of liquorice and its constituents, with particular reference to its value as a pharmaceutical excipient; control and prediction of particle size in ball milling; conductance of emulsions; effect of moisture on porcelain dental fillings; chromatographic techniques applied to crude vegetable drugs; pharmacology and biochemistry of substituted glutarimides; cotton dust and other vegetable dusts in relation to byssinosis; macrocyclic compounds related to steroid hormones; action of bases on chloroform; transformation of glycyrrhetic acid; changes in drug sensitivity in states of avitaminosis.

The Navigation Department occupies the bulk of the two upper floors above the Students' Union. Apart from a small amount of part-time work for yachtsmen, its function is the preparation of serving merchant navy officers for Ministry of Transport Certificates of Competency. It is exceptionally well equipped, as befits the only Navigation Department in a College of Advanced Technology. One special feature is an instrument room, which houses all the aids, electronic or otherwise, which an officer might find on the bridge of a modern merchant ship. A radar simulator (costing £12,000) is now being installed, and this will supplement the training given in the College Radar School at Cardiff docks. Another interesting feature is an extremely compact demonstration set-up, where twenty students can sit around the working bench with not one of them more than 6 ft. from the demonstration.

The communal facilities in this new building include a refectory, kitchen, etc. Above this, and extending through the two upper floors, is the Great Hall. Measuring 80 ft. × 60 ft., this hall, with its gallery, can provide seating for nearly 800. It has a large stage, fully equipped, with ancillary stores and storage space, and two dressing-rooms. A large window at the back of the gallery enables the classroom behind it to be used as a control room for radio and television programmes. Access to the building is arranged in such a way that the great hall can be used without interfering with the rest of the building. The meetings of the British Association for the Advancement of Science, 1960, saw the first use of the Hall, and a number of exhibitions have already been held in it, including one staged by the United Kingdom Atomic Energy Authority.

Finally, reference must be made to the Students' Union. This is a self-contained suite of rooms of some 5,000 sq. ft. and the accommodation includes two lounges, two recreation rooms, a council room, chapel, a music room, a photographic room, together with various offices for the conduct of Union business. Although the Union has always been a very live body, the improvement in facilities has already resulted in a marked development of student activities.

GAS-PHASE REACTIONS OF THE HALOGENS AND THEIR COMPOUNDS

AN informal discussion on "Mechanisms of the Gas-Phase Reactions of the Halogens and their Compounds" was held on March 28 at University College, Cardiff, under the auspices of the Faraday Society. About seventy members and guests were present. The chairman of the first session, Prof. A. G. Evans (Cardiff), welcomed the visitors to Cardiff and introduced the foreign guests. Dr. P. G. Ashmore (Cambridge) then reported further investigations in his detailed study of the pyrolysis of nitrosyl chloride. He showed that the addition of nitrogen dioxide to nitrosyl chloride leads to a fast but rapidly inhibited decomposition. The catalysis was explained by postulating the intermediate formation of nitryl chloride, that is :

$$NOCl + NO_2 \xrightarrow{12} NO_2Cl + NO$$

$$NO_2Cl + M \xrightarrow{14} NO_2 + Cl + M$$

$$NO_2Cl + NOCl \xrightarrow{15} NO_2 + NO + Cl_2$$

The auto-inhibition was explained by reaction of the product nitric oxide with nitryl chloride :

$$NO + NO_2Cl \xrightarrow{13} NO_2 + NOCl$$

Estimates were given for k_{14} with $M = NO_2$ and N_2 and new values had been obtained for k_3 in the reaction :

$$NOCl + M \xrightarrow{3} NO + Cl + M$$

with M = NOCl, NO, N_2 and H_2. One surprising feature of these last results was the very small differences found for the activation energies and pre-exponential factors when M was varied.

The second paper by Prof. R. G. W. Norrish (Cambridge) gave an account of the flash photolysis of nitrosyl chloride in the presence of a large excess of inert gas to ensure isothermal conditions. Many absorption bands were produced in the region 2100–4000 Å. The conditions of the experiments were such that the light causing photolysis was mainly in the region below 2700 Å. Since the N—Cl bond

in NOCl has a dissociation energy of approximately 38 kcal., this corresponds to an excess energy of 70–110 kcal. (at the limit of transmission of quartz). The absorption bands in the spectrum that follows the flash have been assigned to transitions from the ground-state of NO ($X^2\pi$) to excited states $A^2\varepsilon$, $B^2\varepsilon$, $C^2\pi$ and $D^2\varepsilon$, and it was demonstrated that all vibrational levels of the ground-state up to $v'' = 10$ were populated.

Norrish was able to show that at least 50 kcal. of the excess energy of the flash appears as vibrational energy in the nitric oxide molecule, and suggested that this occurs in the primary process, namely:

$$NOCl + h\nu \rightarrow NO^* + Cl$$

Various other possibilities were discounted on energetic or theoretical grounds.

Finally, he suggested that such a process may have a fairly wide generality, and could explain the production of many of the highly vibrationally excited molecules produced in flash photolysis.

Dr. J. H. Thomas (Cardiff) then discussed the gas-phase reactions of methyl chloride, methylene chloride and chloroform with nitrogen dioxide. He showed that the reactions were autocatalytic in nature, and led to complex oxidation products. Two of the products, hydrogen chloride and nitrosyl chloride, had marked catalytic effects when added to the initial reactants, as did the addition of chlorine. He suggested that the autocatalysis resulted from the production of chlorine atoms by reactions such as:

$$HCl + NO_2 \rightarrow Cl + HNO_2 \quad (1)$$

and

$$NOCl \rightarrow NO + Cl \quad (2)$$

followed by hydrogen abstraction by the chlorine atoms, for example:

$$CH_3Cl + Cl \rightarrow CH_2Cl + HCl \quad (3)$$

The final products were explained by a mechanism involving further reactions of the radicals produced in (3). The reactions of the three chloromethanes were compared, and it was explained that carbon tetrachloride was unreactive until much higher temperatures were reached.

In the second session, with Prof. R. G. W. Norrish in the chair, two papers on aspects of the photochlorination of chlorinated ethylenes were presented by Prof. F. S. Dainton (Leeds) and Prof. P. Goldfinger (Brussels). These reactions all involve chains initiated by chlorine atoms via :

$$A + Cl \underset{2}{\overset{1}{\rightleftharpoons}} ACl$$

where A is an olefine. The radical ACl finishes in the main as ACl_2 and various chain-termination steps may occur. Both groups of workers used experiments with intermittent light to find the Arrhenius parameters for the various chain steps.

Dainton has studied the olefines $C_2H_nCl_{4-n}$, n = 0–4, with the exception of vinylidene chloride. Reaction (1) is very exothermic for these olefines, hence the ACl radical should have considerable energy and may be regarded as an excited molecule ACl^*. The Arrhenius parameters for reaction (2) should then be quite different from those for the decomposition of a normal ACl radical. This was found to be so for A = vinyl chloride, where E_2 = 0·7 kcal. mole^{-1} compared with E_2 = 22·0 kcal. mole^{-1} from pyrolysis studies.

Goldfinger has studied trichloroethylene to find out if reaction (1) above gives the symmetric ($CCl_2.CCl_2H$) or asymmetric ($CCl_3.CHCl$) radical. The method used involved the photochlorination of trichloroethylene in the presence of either sym. or asym. tetrachloroethane (since hydrogen abstraction by chlorine atoms from the last two substances will give the sym. and asym. radicals referred to above). An analysis of the kinetics of the reactions of these mixtures showed that reaction (1) gives the sym. radical, that is :

$$CHCl = CCl_2 + Cl \rightarrow CHCl_2 - \dot{C}Cl_2$$

The activation energy for the reverse of this reaction was found to be 19 kcal. mole^{-1}, which is much larger than the value of 6·6 kcal. mole^{-1} found by Dainton for the same reaction. Goldfinger was unable to find evidence of the formation of excited ACl radicals.

After lunch, Prof. E. C. Kooyman (Leyden) was chairman and the first speaker was Dr. E. Whittle (Cardiff), who described the photosensitized brominations of methanol, ethanol, formaldehyde and acetaldehyde. The main purpose of these investigations was to obtain information about the strengths of certain of the bonds in the molecules. The products of the reaction were identified and explained in terms of chain reactions involving bromine atoms. Dr. Whittle discussed in detail the photobromination of methanol and its kinetics under varying conditions, which resulted in different chain termination processes. The reaction $Br + CH_3OH \underset{2}{\overset{1}{\rightleftharpoons}} CH_2OH + HBr$ was found to have an activation energy E_1 = 6 kcal. mole^{-1}. This gives $\Delta H_1 \leqslant 4$ kcal. mole^{-1} and hence $D(H—CH_2OH) \leqslant 92$ kcal. mole^{-1}. The CH bond in methanol is thus considerably weaker than in methane, for which $D(H—CH_3)$ = 102 kcal. mole^{-1}.

Dr. J. M. Tedder (Sheffield) then discussed the fluorination, chlorination and bromination of the butanes and normal and secondary butyl halides. For unsubstituted butanes the activation energy for abstraction of hydrogen atoms by different halogen atoms are of the order : fluorination 100 cal. mole^{-1}, chlorination 1,000 cal. mole^{-1}, bromination 10,000 cal. mole^{-1}. In all cases the activation energies decrease for different hydrogen atoms in the order tertiary < secondary < primary.

The introduction of substituent halogen atoms into butane has a considerable effect on the rates of halogenation of sites adjacent to the substituent. The previously accepted assertion that a substituent halogen atom directs attack by chlorine atoms away from itself was shown to be incorrect, for example, in the chlorination of 2-chlorobutane the dichlorobutane formed in the largest amount was 2.2-dichlorobutane. For brominations similar effects were found, but in the fluorination of 2 halobutanes only slight attack occurs at the 2- position. The experimental results were interpreted in terms of the Evans and Polanyi picture of the transition state.

The final session with Prof. F. S. Dainton in the chair was opened by Prof. Kooyman, who described the results of a study of the products of the chlorination and bromination of aromatic materials in the gas phase. Chlorinations were carried out at temperatures between 300°–450° C. and brominations between 400°–500° C. Typical reactions were between bromine or chlorine and diphenyl ether, fluorobenzene, chlorobenzene and benzonitrile. The striking feature

of all these reactions is that substitution occurs with major products being the meta-isomers. This is in complete contradiction in all cases to liquid-phase halogenations where *ortho/para* substitution predominates.

Kooyman said that a radical mechanism alone could not readily explain the specificity of the results obtained and put forward the novel suggestion that the reactions occurred through an ionic intermediate, that is :

$$X_2 \rightarrow 2X$$
$$X + ArH \rightarrow [ArH^+][X^-]$$

A proton is then lost from the position with highest positive charge, that is, the *meta* position :

$$[ArH^+][X^-] \rightarrow Ar + HX$$
$$Ar + X_2 \rightarrow ArX + X$$

This mechanism provoked a lively discussion, and the outcome of Prof. Kooyman's present work on the kinetics of these reactions is awaited with interest.

Finally, Dr. B. A. Thrush (Cambridge) described spectroscopic studies of bromine atom recombination. The studies employed the technique of flash photolysis to study the recombination of bromine atoms in excess argon to determine whether the bromine molecules formed in the recombination are produced in an electronically excited state or with excess vibrational energy. No evidence for the former was found but absorption spectra obtained during the recombination showed an appreciable increase in the population of the lower excited vibrational levels of the ground-state, notably $v'' = 1,2,3,4$ and 5.

The distribution of this energy corresponded to temperatures much higher than those to be expected from the amount of energy absorbed from the flash.

The papers presented stimulated a lively discussion throughout and thus ensured the success of the meeting.

J. H. Thomas
E. Whittle

AUTHOR AND PUBLISHER

PROBLEMS arising in connexion with the publication of scientific books were discussed at a recent meeting of the Scientific Publications Council held at the Ciba Foundation, London. Prof. G. W. Harris was in the chair.

Mr. David Dickens (Pitman Medical Publishing Co.) said that the publication of scientific books is not a lucrative business. Scientific books are generally written for the specialist, and consequently the market for them is small. A publisher of scientific text-books must maintain a staff of highly qualified editors and sub-editors, generally with science degrees, who work together with the authors to get the manuscripts into shape for publication. This is often a long process involving heavy investment: one book alone might cost £4,500 to produce and may take many years to bring back the outlay. The publisher contributes, as indicated by his 'imprint', prestige, knowledge of the field and 'know-how'. He has the same interest as the author in getting a book published as quickly as he can.

Ownership of Copyright

Mr. Dickens stated that the copyright is the author's as soon as he puts pen to paper. The publisher is generally better able to deal with selling the rights than the author, and he should do this in the author's name. Difficulties may arise if a book is written by joint authors who later decide to part. Mr. D. M. Neale (Oxford University Press) believed that it is often more convenient for the publisher to hold the copyright in order to protect the author's interests, although this is not necessarily a good general rule. His firm has been taking legal action to stop a book being pirated in Hong Kong: it could not have done this if the copyright were in the author's name, since the author was in Europe doing research. The cost of fighting an action of this kind is enormous and is all borne by the firm. When an institute publishes a series of volumes, the copyright is sometimes held by the institute: otherwise the Oxford University Press prefers to keep the copyright and return to the author whatever they may get from sale of the rights. Difficulties can arise if a publisher holding the copyright is taken over by another firm: the author has generally no defence against that. Miss M. E. Barber (Society of Authors) said that some publishers never take the copyright, but others do claim it, often in the first clause of the contract. The copyright includes translation, broadcasting, television, film, drama, digests and paperback editions. The Society of Authors takes the view that the publisher does not need the copyright: he should negotiate the rights as the author's agent. Scientists often regard writing as a side-line, and they do not take the trouble they should over contracts: publishers therefore treat scientists in a way in which they would not treat other authors. Only if piracy were rife enough to be an overriding consideration would it be justified for the publisher to hold the copyright.

Mr. A. L. Bacharach (Nutrition Society) said that the author of a paper in a scientific journal is in a different position from the same author when writing a text-book. The copyright of a scientific paper is owned by the author, unless he has specifically assigned it to a body, such as a learned society: but recently it has been suggested that the copyright of a paper belongs to the employer of the author. Dr. E. A. Moelwyn-Hughes (Cambridge) said that some countries still take no notice of copyright. When in the U.S.S.R. he had discovered by chance that 20,000 copies of a book by him had been distributed there, but he had received no royalties from the Russians. He had heard that there was also a Japanese edition, from which he also had no royalties. It is generally understood that an author can quote material from another publication so long as he gives the source and reference, but some publishers object to this. What is the legal position ? Miss Barber replied that quoting from another publication is often done by scientists, but it is an infringement of copyright to quote "a substantial part" of a copyright work without permission. Even summarizing a publication of another author or publishing an abstract is an infringement of copyright if done without permission.

XUM

Contractual Relationships

Prof. G. V. R. Born (London) said he knew of scientists who had experienced difficulties with their publishers. How can scientists be warned about publishing difficulties? Contracts often contain an arbitration clause which may delay decision in disputes; would it be better for authors if there was no such clause? Can a publisher be asked for an audited account? Miss Barber said that an author is entitled to detailed royalty statements and that, if he had good reason to doubt their accuracy, he had a common law right to inspect the publishers' books of account, though this was a little cumbersome to enforce. The name of an unreliable firm cannot be published openly or broadcast; but a name can be omitted from a list of recommended firms. Mr. Bacharach said that the good publisher presents no difficulty: contracts are necessary, because there are bad publishers who try to manipulate an agreement to the author's disadvantage. Sometimes contracts contain a clause giving the publisher the right to call on the author to prepare a new edition; they should include also a clause requiring the publisher to let the author make such revisions as he may wish to a new issue. This is particularly desirable in scientific broadcasting; the B.B.C. has agreed that there should be no repeat after six months without giving the author a chance to revise.

Dr. Moelwyn-Hughes referred to an eminent scientist who had been cheated by a publisher. Something needs to be done in the scientific world to combat the recent development of mushroom firms that are financially slick and trade on the business incompetence of the scientist. Dr. D. Richter (Carshalton) said that British publishers have built up a high reputation in the past, so that authors have come to trust their publishers as they would their doctors or solicitors, but recently publishers of a different type, interested mainly in quick profits, have entered the scientific field. There have been instances when the publisher has sold rights without informing the author, when inaccurate royalty statements have been given and when scientists have received no royalties at all from sales of their books. Unfortunately the reliable old-established firms tend to be slow and do not always appreciate the special requirements of the scientist. The mushroom firms are exploiting the good faith of the scientific writer and giving British publishers a bad name. Prof. G. W. Harris said there is a need especially for the younger scientists to be informed of the situation.

D. Richter

SOUTH-EASTERN UNION OF SCIENTIFIC SOCIETIES ANNUAL CONGRESS

At the invitation of the Haslemere Natural History Society and the management committee of the Educational Museum, the South-eastern Union of Scientific Societies held its sixty-sixth annual congress at Haslemere during May 26–28, under the presidency of Prof. S. W. Wooldridge. The Council's choice of the theme for the congress of "The Changing Countryside" proved successful, allowing the lecturers full freedom in the presentation of their subjects and of focusing attention on the need for preservation and the desirability of intelligent management in Nature conservation. The delegates were welcomed by Dr. Rolston, chairman of the management committee of the Museum.

The young naturalists' evening included a brains trust with questions answered by a panel of experts, under the chairmanship of Maxwell Knight, followed by the screening of a film "Journey into Spring", introductory to the visit to Selborne.

The presidential address was given by Prof. S. W. Wooldridge, whose subject was "The Land and Man in the Western Weald". He commenced by emphasizing that, although the land existed before man, it was not an environment until man entered on it and reacted to it. The notion that the Weald is a denuded simple anticline has existed for some 150 years, from the time of John Fairey, who also introduced the use of the term 'denudation'. His observations of the area were made from the top of a stage-coach in the days of leisurely travel. It is now known that between the south coast and the Thames valley there are six anticlinal crests, somewhat resembling corrugated iron in form. For convenience of understanding, Prof. Wooldridge defined the country west of Stane Street as the Western Weald, wherein the Hythe Beds suddenly expand to form a major feature. It was important to remember that at one time the Chalk stood 2,000–2,500 ft. above the present land-level near Haslemere. Dealing with the influence on subsoil and soil types, it was pointed out that the intermediate and loamy soils are not derived from one particular formation, but have different soil parents. Our ancestors chose the Upper Greensand at the base of the chalk as good growing land, the best arable ground. It should be remembered that the dry valleys were not dry even in Romano–British times. Prof. Wooldridge next dealt with the succession of occupants. During Mesolithic times, about 6,000 B.C., when the climate was wetter than at present, the area on the Surrey–Sussex border was occupied. Evidence of man's later activities, during Neolithic times, had been found in the ditches at the Trundles. The following early Bronze Age had the same type of life and culture, and it was not until the late Bronze Age that the real cultivation of the land began. There is evidence of industry and trade during the Iron Age, 500 B.C.; the so-called hill forts are really the beginnings of cities on the hills. A later stage was the creation of the village communities, about A.D. 600, located by the early Saxon place-names, often those of pagan deities. The last stage is indicated by the distribution of the Domesday vils, the parish boundaries showing a remarkably even pattern, especially along the edge of the Tertiary beds.

The zoological section was addressed by John Sankey, on "Invertebrate Life in South-east England". He has for 14 years been fighting a campaign for greater investigation of the lesser-known invertebrate animals. The butterflies and beetles had been studied well enough, but such groups as centipedes and spiders need far more attention, especially observational work on their life-history and ecology.

Unless this can be done now, the changing countryside will eliminate them before they can be studied. Foam, muck and dirt are polluting many of their habitats. Specimens of the lesser-known invertebrates do not make a great display, but the need is for the collection of information rather than of specimens. Information is especially required of the habits of the fresh-water sponge, flat worms, millipedes, spiders and molluscs; the life-cycles of many are not known. The only locality in the south-east of England for the raft spider is at Frensham bogs, and it would be a great pity if the area is drained, causing its extinction. Mr. Sankey emphasized the necessity for a true understanding of conservation, not by leaving areas alone, but by proper management by people who know what they are doing.

The address to the botanical section was given by J. E. Lousley on "The Changing Flora of South-east England". He said that conditions are changing constantly and rapidly, but all changes are not necessarily bad; things need not always remain as they were when one was young. In a progressive world one must be progressive, and it is well to remember that the quarries and sandpits excavated by our grandfathers are now some of our best habitats. There was considerable upheaval by the building of railways, but there has been good recolonization of the railway embankments. The canals, many of which are now abandoned, are rich in aquatic plants, some of which had been introduced by the barges, and the filled-in canals provide habitats for many species. The greatest losses have been through building extensions; that around London has destroyed many habitats, while drainage has reduced the number of boggy areas. The indiscriminate dumping of rubbish is deplorable; the example at Mitcham Common was to be avoided. Changes are to be seen in the village ponds (no longer required for animals), which have now become the receptacles for rubbish. A further undesirable change is brought about by the use of the ground by the Army for tank training, and the carelessness of the public is a constant threat, by fire, on heathlands. Great changes in the vegetation have been brought about by the reduction of grazing and the deplorable elimination of the rabbit, resulting in the vast increase of scrub. It is now necessary for a conservation corps to be constantly clearing the scrub to maintain the survival of the interesting plant life.

The general interest of the public has increased, particularly in the British flora, and this aids the propaganda for the need of preservation and conservation.

Miss Phyllis Bond addressed the delegates on "Birds in a Changing World". The change in south-east England was swifter than in most areas, owing to its proximity to London, increasing greatly since the beginning of the century. The spread of residential building may increase the bird population, gardens providing a variety of habitats, but the occupants are the so-called common birds and the need is to provide suitable habitats for the rarer species in danger of elimination. One of the hazards of progress is that a large part of the resident population is not indigenous and is always tidying up, thus removing the habitats necessary for some birds. Nature and man have from the earliest times moulded the shape of the countryside, and man's effort is often not to its advantage. Miss Bond regretted the passing of the rabbit; the change in the nature of the herbage has been to the disadvantage of many birds, depriving them of their usual nesting sites and reducing the insect population, which provided their food. She deplored the practice of spraying road-side verges and hedgerows with poisonous compounds, especially between the months of April and August. The shrubs and herbage which were the breeding places of many birds are either killed or starved; there is a chain of deterimental reaction the ultimate result of which cannot be perceived. Poisoned seed is also responsible for much injury, for not only are birds and animals killed by directly taking the seed, but also their food of worms and other invertebrates carries the poison and thus adds to the casualties.

The attitude of the public towards birds has changed for the good; the shooting of birds and the collection of eggs are now regarded as belonging to past generations.

Dr. J. F. D. Frazer, conservation officer for England of the Nature Conservancy, chose as the title of his address, "Problems of Managing Nature Reserves". The Royal Charter granted to the Nature Conservancy included a clause enabling it to acquire and manage nature reserves. Land left alone becomes a wilderness so the principles of management have to be studied and applied. First, the type of nature reserve desired has to be determined, and the drawing up of a management plan is the work of two or three years. The past history of the area has to be studied, its geology, the arrival of man, up to its present state. The climate and existing species have to be studied; it has to be decided what is wished to be done and how it is going to be achieved. In some cases the public can be allowed access without harm being done, but when sand dunes are the subject the public must be kept off. An area may need to be managed correctly, as at Blean Wood, Kent, for the protection of one butterfly or as at Wye for the preservation of an orchid population. The desirability of introducing 'foreign' animals and plants or the re-introduction of those that have become extinct has to be discussed and the good relations with the owner of adjacent property encouraged.

Dr. Frazer showed slides of areas taken from the same point yearly during 1954–60 showing clearly the evil results of the extermination of the rabbit, which controlled the growth of ragwort, gorse and the scrub. Without grazing, the only way to reproduce the former condition is by cutting, and a corps of conservationists is required to work on this, under the direction of a conservation officer, who determines the areas to be cleared, the amount necessary and the regularity of the period of clearance to ensure success. Favourable weather conditions enabled the full programme of excursions to be carried out. The archæologists visited Selborne Priory and church, and a general natural history party after visiting 'The Wakes' at Selborne continued to Nore Hill for the Chalk flora. Botanists and zoologists went to the Juniper Hall Field Centre, Box Hill, and the surrounding countryside for the Chalk flora and invertebrates; particular interest was shown in the trap-door spiders at their only known locality in south-east England.

The excellent arrangements for the congress were undertaken by a local committee with John Clegg as secretary.

The sixty-seventh annual congress of the Union will be held at Colchester during June next year, and it is hoped that the meeting will provide an opportunity to stress the importance of the natural sciences in the curriculum of the new university to be established there.

F. J. Epps

PARASITE BIOLOGY

THE third spring meeting of the Parasitology Group of the Institute of Biology was held at Wills Hall, Bristol, during April 11–13. The meetings were well attended, and a number of visitors from overseas were present. There can be no doubt that this annual meeting held at a provincial centre has become established firmly in the activities of the Group. An important item in the programme was the address by an invited speaker. This item was introduced last year at Liverpool when Prof. T. H. Davey took as his subject "Parasites as Enemies and Allies". It proved a very useful focus of interest, and this year Prof. C. Read was invited to speak. He gave as his title "The Host-Parasite Interface: Molecular Basis of Parasitism". Prof. Read is professor of biology in the Rice Institute, Houston, Texas, and is at present on sabbatical leave working at the Molteno Institute, Cambridge. He began by discussing parasitism as part of a broad definition of 'symbiosis' considered as a function of the intensity of the field of association established between organisms, and of the internal and external resistances surmounted in its establishment. The parasite comes to depend on the host for the maintenance of homeostasis, and the regulation of homeostasis of a parasite intimately involves a region in space and time which can be designated 'the host-parasite interface'. The host, however, is not a passive environment, and interaction plays an important part in this concept of parasitism. The primary exchanges at the interface are chemical, and Prof. Read proceeded to discuss examples of these obtained from work with which he has been associated in the United States. He concluded by suggesting how host-parasite specificity and pathology might be discussed in terms of chemical exchanges.

The general sessions were devoted to a series of 19 short papers, each being followed by a period of discussion. This type of meeting is useful because it gives an indication of the fields in which the main research is taking place and provides an opportunity for discussion of its progress and the conclusions reached.

The growing interest in marine parasitology was reflected in two papers by Z. Kabata (Marine Laboratory, Aberdeen) and H. H. Williams (University College of Wales, Cardiff). Dr. Williams had examined a number of elasmobranchs caught in British fishing areas and recovered the tetraphyllidean cestodes which they contained. A high degree of host-specificity exists in this group, and, although there are still many unidentified species, a very interesting discussion was given of the physiological, ecological and morphological factors which contribute towards restricting a species to a particular host. Dr. Kabata based his discussion on the parasitological examination of some 500 whiting and haddock and presented some data relating the age of the fish to the parasitic fauna which it supports.

Temperature and climatic conditions have an important influence on the level of helminthic infestation in domestic animals. H. D. Crofton (Bristol) has devised a technique for accurately observing the rate of development of nematode eggs and larvæ from sheep. The data, which he has obtained on the limits of temperature tolerance of different species and the effects of temperature on the rate of development, provided the basis for a useful discussion on the ecology of the parasites in relation to domestic stock. The importance of temperature and moisture-levels on different stages in the life-cycle of *Fasciola hepatica* was discussed by C. B. Ollerenshaw (Central Veterinary Laboratory, Weybridge), with particular reference to the incidence of fascioliasis in Anglesey. Dr. Ollerenshaw was able to correlate the incidence of the disease in this area, which was particularly high during 1958, with meteorological data obtained during the preceding year.

The significance of the trypanosomatid kinetoplast figured in the discussions by three speakers. J. R. Baker (London School of Hygiene and Tropical Medicine) and K. Vickerman (University College, London), in a joint paper, showed a series of electron micrographs of insect and vertebrate trypanosomatids, and the possible functions of the kinetoplast were reviewed with particular reference to kinetoplastic and akinetoplastic strains and to the life-history of the species studied. When trypanosomes (*T. gambiense* and *T. rhodesiense*) are exposed to dilute solutions of acriflavine they become photosensitive and are rapidly killed by light. The kinetoplast of the sensitized trypanosome fluoresces intensely in ultra-violet light, but experiments by P. J. Walker (National Institute for Medical Research, Mill Hill) have indicated that this organelle is not the only site of the photosensitivity.

The role of arthropods as vectors of parasitic infections was discussed in the course of three papers. D. S. Bertram and R. G. Bird (London School of Hygiene and Tropical Medicine) described the subcellular morphology of *Aedes aegypti* with the aid of a series of electromicrographs. This study was particularly concerned with the transmission of Semliki Forest virus, and the presence of virus particles within the cells was correlated with the infectivity of the insects. These workers also suggested that the complexity of the membranes surrounding the anopheline ovary reduces the possibility of the transmission of infection through the mosquito egg. In ticks, however, the membrane structure is simpler, and transovarian transmission of parasitic infections therefore can take place more easily. Transmission in this way of *Babesia divergens* by larvæ of *Ixodes ricinus* following the feeding of adult ticks on infected calves had been observed by Davies and Joyner in the course of a series of transmission experiments carried out at the Central Veterinary Laboratory, Weybridge. The feeding mechanism by which *Ixodes ricinus* obtains infected blood was the subject of an investigation by E. Sutton (King's College, London). Histological preparations were shown to illustrate the digestion of the host tissue which takes place around the inserted mouth-parts. Spectrophotometric measurements of the hæmoglobin content of individual ticks indicate that ticks feed on varying amounts of non-blood and blood tissue.

Parasites in relation to natural fauna were also discussed. A. M. Dunn (Veterinary School, Glasgow) has recorded the intestinal helminth parasites of the

three species of wild deer on Rum in the Inner Hebrides. This paper was notable for some very beautiful illustrations of the habitats. The rigour of a littorine habitat was apparent in the discussion by B. L. James (Aberystwyth) of the development and life-history of *Parvatrema homoeotecnum* n.sp., a gymnophalline trematode which parasitizes the gastropod mollusc, *Littorina saxatilis* and oyster catchers. This parasite shows several specialized and primitive features. The perch and pike in Llyn Tejid in Merionethshire are the hosts of a pseudophyllidean cestode, *Triaenophorus nodulosus*. By taking regular nettings, J. C. Chubb (Liverpool) was able to obtain data relating the incidence and development of the parasite to the growth of the fish. Also following this theme of seasonal incidence, but with quite a different parasite, W. N. Beesley (Weybridge) discussed the development and migration of *Hypoderma bovis* and *H. lineatum* in domestic cattle.

Work during recent years has shown that parasitology is a fertile field for the application of new laboratory techniques. Thus, cytochemical techniques were used to characterize the functions of the male accessory and reproductive glands of *Argas persicus* (R. J. Tatchell, Cambridge), and the pseudosuckers and holdfast organ of the trematode *Diplostomum phoxini* (D. L. Lee, Cambridge). The nutritional requirements of the latter have been the subject of *in vitro* cultural studies by C. A. Hopkins and M. O. Williams (Glasgow). In the field of immunology, agar diffusion techniques have been applied to such widely differing subjects as *Eimeria* infections in fowls (M. E. Rose and P. L. Long, Houghton) and schistosomes in monkeys (S. R. Smithers, National Institute for Medical Research). Despite the application of new techniques, parasitology still occasionally requires systematic descriptions, an example of which was the paper by S. T. Fernando (Cambridge) dealing with the developmental stages of *Mecistocirrus digitatus*, a nematode found in the abomasum of cattle in Ceylon, India and the Far East.

The proceedings of this meeting will not be published in detail. The spring meeting in 1962 is to be held in Cambridge during April 3–5.

L. P. JOYNER

WATER RESOURCES IN WALES

LARGELY as a result of the water supply difficulties of 1955 and 1957, the Minister of Housing and Local Government (who is also Minister for Welsh Affairs) appointed in February 1958 a committee to advise him on the use and conservation of water resources in Wales. The Committee has already expressed interim views in February 1959 on the Milford Haven barrage scheme (which it favoured), and has recently published its first full *Report on the Water Resources of Wales**.

This report is a notable advance forward in our knowledge of the Water Resources of Great Britain and in their proper employment. The Minister indicated to the Committee that the most urgent problem was to assess the water resources in relation to foreseeable demand, and three guiding questions were posed: (*a*) What are the water resources? (*b*) What is the exploitable surplus? and (*c*) How can such surplus best be employed?

The Committee consisted of persons from different parts of Wales with interests in hydrology but not themselves engineers or technical experts. The report, as a result, considers not only the hydrological survey of Wales carried out by the Ministry's engineers in 1957 but also social, economic and cultural aspects of the problems encountered. The hydrological survey (not generally available) gives detailed estimates of the maximum supply of water which could be obtained from Wales on the assumption that hydrological considerations are the sole governing factor. The Committee, however, examined more than 180 suggested reservoir sites in the light of (*a*) geological structure, (*b*) agricultural interests, (*c*) existing communities, (*d*) amenities, and (*e*) cost. The sites were then placed in one of four categories: '*A*' sites, to which no objection could be seen; '*B*' sites, where a moderate degree of objection was foreseen; '*C*' sites, development of which could only be envisaged if the need for water in the country as a whole were to become over-riding; and lastly' sites rejected as completely unsuitable.

As the Committee also considered the various resources in relationship to conventional impounding reservoirs, regulating reservoirs, estuarine reservoirs and direct abstraction, it will be immediately apparent that there is no clear-cut and definite answer to the first question: "What are the water resources of Wales?". Appendix 8 of the report, however, sets out the various possibilities: these, on the Committee's definition, range from a minimum of 1,195 million gallons a day to a maximum of 2,895 million gallons a day.

These resources are next considered in relationship to the demand for water. Here the Committee has not only considered the present situation but also has looked ahead into the 'foreseeable future'. This has been taken, despite the hazards of extrapolation, as a period of 30 years. For this purpose Wales is divided into five geographical zones—south-west, north-west, north-east, south-east and central. In only one of these zones, the south-east, has the Committee found serious prospective deficiency of water: in all the others there is not only an adequate amount of available water but also an exploitable surplus. Ample allowance has been made in these calculations for a continued expansion in the Welsh economy and for increased demands for water. The Committee estimates that, taken as a whole, the Principality has an exploitable surplus of resources over demand of between 235 and 800 million gallons a day according to the system of development adopted (this is seen in perspective when it is recollected that public undertakings in England and Wales at present supply some 2,400 million gallons a day).

The Committee suggests that that part of the surplus which can be most easily used to supply areas in England which are short of water is to be found in the upper basins of the Severn, Wye and Dee (the 'central zone'). This is an area of heavy rainfall and very low population with limited local demands on

* Welsh Advisory Water Committee. *Report on the Water Resources of Wales*. Pp. iv + 93. (Cmnd. 1231.) (London: H.M.S.O., 1961.) 5s. net.

the water resources. The report stresses that this should be achieved by means of regulating reservoirs such as that proposed in the Tryweryn scheme. This type of reservoir regulates the flow of water down the river, and water is then abstracted from the lower reaches: there is no pipe-line as with the impounding reservoir.

The Committee raises an important point in the suggestion that rural areas where large reservoirs have been sited have not always received adequate economic returns. They believe a case exists for a system whereby material benefit should be received by such areas in addition to the extra revenue available in the form of rate income. Adequate compensation for displaced residents and disrupted farm-economies would also ease the transition from traditional types of land use to water storage. A realistic approach on these lines would help to create a better understanding between the city and town dwellers and the inhabitants of the rural catchment areas.

The report is full of valuable statistical data, and provides for the first time an indication, in the light of economic, social and cultural considerations, of the hydrological situation within the Principality in relationship to present and future needs of water supply. A clear and concise geographical account is also given of each of the five zones demarcated (although the authors of the report have somewhat oddly listed the physical geography as 'geography'; actually the whole of each of the respective chapters falls within the scope of a geographical consideration). As a work of reference and as a guide for future policy decisions the report will be invaluable.

Water in Wales may also raise from time to time 'political' problems regarding the 'export' to England. This aspect is also considered by the Committee. It believes that, in a period when water will become relatively more scarce, Wales will have many advantages for water-seeking firms and for agricultural irrigation. While noting that public opinion in Wales would not take kindly to the idea of a wholesale export of water without reference to long-term needs within Wales, the Committee is able to conclude that Wales has such an abundant rainfall that there is, and will continue to be, a large exploitable and 'exportable' surplus of water if the resources were properly developed by the construction of suitably sited regulating reservoirs. W. G. V. Balchin

CHEMICAL COMPOSITION OF PRECIPITABLE WATER VAPOUR OVER THE UNITED STATES

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DATA on annual average amounts of precipitable water vapour in the atmosphere over several stations of the United States and up to 525 m.b. have recently been published by Reitan[1], and it appeared to be of interest to us to attempt a correlation between these data, Junge's rain-water content analyses[2,3] and the total precipitation values. It was thought, in particular, that further evidence of a connexion between precipitation and such occurrences as may induce changes and activation[4] in aerosols could be obtained in this way. Since about half the stations selected by Reitan for his work coincide with points which were used by Junge, there appeared to be a good possibility of gaining further insight into natural nucleation phenomena.

If the precipitable water vapour π is taken as a potential measure of the water stored in the atmosphere, and other parameters are maintained constant, an increment of precipitation R with increase of π will depend on an effective value of π itself, and therefore on the difference:

$$\alpha\pi - \beta R$$

α and β are constant for the purpose of this article (see later); more generally α is a number which depends on temperature, while β has the dimensions of time.

Therefore:

$$\frac{dR}{d\pi} = K(\alpha\pi - \beta R) \qquad (1)$$

where K is a constant.

When $\pi = 0$, $R = 0$; equation (1) integrates to:

$$R = \frac{\alpha}{\beta}\left\{\pi - \frac{1}{K\beta}\left[1 - \exp(-K\beta\pi)\right]\right\} \qquad (1a)$$

The derivative of (1a) with respect to π can also be written:

$$\frac{dR}{d\pi} = \frac{\alpha}{\beta}\left\{1 - \exp(-K\beta\pi)\right\} \qquad (1b)$$

Thus, it is significant that for very high precipitable water vapour contents of the atmosphere (1b) approaches α/β, while it is near to zero for very small values of π.

Clearly, the 'tendency of π to precipitate' will depend considerably on temperature. If, however, differences in vertical temperature gradients from station to station are assumed to be such as not to have much influence on the data at hand, it becomes readily possible to correct π for these effects. Rather than make corrections on the basis of the $\pi - T$ empirical relationship and the 'potential temperature' formula, we used an empirical latitude correction. A plot of π against geographical latitude for lower stations (Fig. 1) gives an approximately straight-line relationship, on the basis of which Reitan's data of precipitable water vapour were all reduced to a latitude of 35° N. by means of multiplication by the corresponding values of $(\pi_{35°}) : (\pi_{\text{lat.}})$ which were obtained from the straight line derived by the method of least squares. This procedure results in a plot given in Fig. 2, where the corrected points are seen to fall into a type of pattern which might be expected to be in agreement with equation (1a).

When the exponential in (1*a*) is expanded in series, the expression becomes:

$$R = \alpha\left(\frac{K\pi^2}{2!} - \frac{K^2\beta\pi^3}{3!} + \ldots\right) \qquad (2)$$

if K is now assumed to depend simply on the quantity of an active chemical species in the atmosphere, and if that quantity were taken, for the purpose of this communication, to be proportional to the concentration Ch of the species concerned in the water that dissolves it, multiplied by the volume of precipitation itself:

$$K = aChR$$

substitution into (2) gives:

$$\pi^{-2}(Ch)^{-1} = \alpha\left(\frac{a}{2} - \beta\frac{a^2}{6}(ChR\pi) + \ldots\right) \qquad (2a)$$

Similarly, if the ratio Na : Cl (ref. 5) is assumed to influence K so that:

$$K = a\frac{\mathrm{Na}}{\mathrm{Cl}}$$

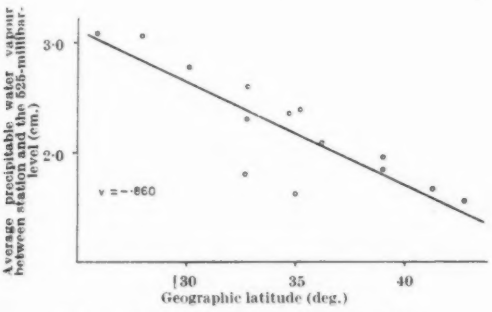

Fig. 1. United States stations; altitude 0–580 ft.

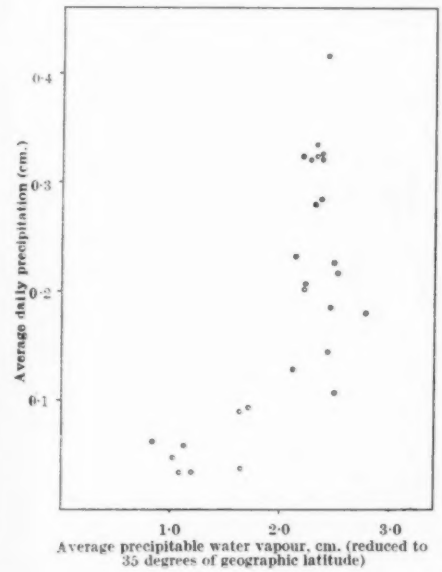

Fig. 2

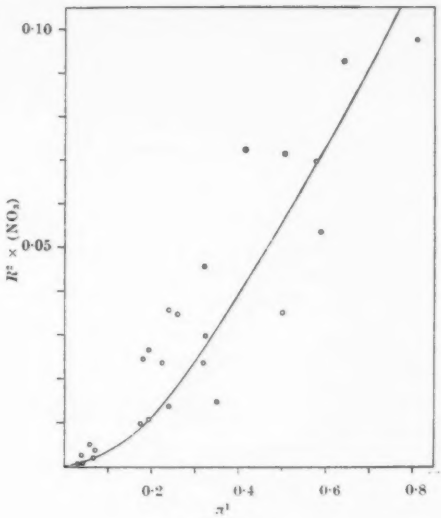

Fig. 3

then equation (2) becomes:

$$\frac{R}{\frac{\mathrm{Na}}{\mathrm{Cl}}\pi^2} = \alpha\left(\frac{a}{2} - \beta\frac{a^2}{6}\frac{\mathrm{Na}}{\mathrm{Cl}}\pi + \ldots\right) \qquad (2b)$$

By means of (2*a*), (2*b*) correlation tests: $(\pi^{-2}(Ch)^{-1}) - ChR\pi$ can be carried out; linear correlation coefficients were computed for the following:

NO_3; Na; K; SO_4; Cl; NH_4; Ca; $\frac{\mathrm{Na}^2}{\mathrm{Cl}}$; $\frac{(\mathrm{Na}+\mathrm{K})^2}{\mathrm{Cl}}$;

Na × NO_3; $\frac{\mathrm{Na}}{\mathrm{Cl}}$; $\frac{\mathrm{Na}+\mathrm{K}}{\mathrm{Cl}}$

(having expressed Ch in gram-equivalents × 10^3 l.$^{-1}$, π and R in cm. and cm. × day^{-1} respectively, and using equation (2*b*) for the last two functions listed).

The following values are worthy of note:

Ch	NO_3	$\frac{\mathrm{Na}}{\mathrm{Cl}}$	$\frac{\mathrm{Na}+\mathrm{K}}{\mathrm{Cl}}$	SO_4	NH_4	Ca
r	−0·77	−0·57	−0·60	−0·51	−0·58	−0·46

All others gave $0 > r > -0{\cdot}4$; calculation of the correlation coefficient between $R\pi^{-2}$ and π (equation 1), where the 'salt effect' was neglected, yields $r = 0{\cdot}21$.

As can be seen, NO_3 is the chemical species that fits equation (2*a*) most closely. Equation (1*a*) can be written in this case, after substitution:

$$R^2NO_3 = \frac{\alpha}{\beta}\left\{\pi' - \frac{1}{a\beta}\left(1 - \exp(-a\beta\pi')\right)\right\} \qquad (3)$$

where

$$\pi' = NO_3R\pi$$

$a\beta$ can be obtained in first approximation from the linear regression parameters of a plot of $\pi^{-2}NO_3^{-1}$ versus $NO_3R\pi$ (equation 2*a*) since its value is:

$$\frac{3\,\text{gradient}}{\text{intercept}} = 3{\cdot}6 \pm 0{\cdot}5$$

Using this value, α/β can be computed with good approximation from equation (3); we find:

$$\frac{\alpha}{\beta} = 0{\cdot}209 \pm 0{\cdot}015$$

This now enables a check of $\alpha\beta$ to be carried out; the method of consecutive adjustments, using the *S.D.* of equation (3) as a monitor, yields a value of $\alpha\beta$ in agreement with that obtained previously.

Fig. 3 illustrates a plot according to equation (3) and the calculated line. The standard deviation of this function is found to be:

$$S.D._{R^2XO_3} = 0{\cdot}013$$

whereas, introducing the constants computed into equation (1) we calculate the value of:

$$S.D._R = 0{\cdot}10$$

We are now in the process of extending the computations based on the use of nitrate to the four seasons during 1955–60. While the results obtained will be the subject of a future publication, it can now be stated that the same correlation appears to exist throughout, and that consistency appears to be higher during the warmer seasons than in the cold ones.

We are indebted to Prof. V. Caglioti for his help and encouragement, and to Prof. G. Fea for the interest he has shown in our work.

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CRYSTALLIZATION IN WOOL KERATIN

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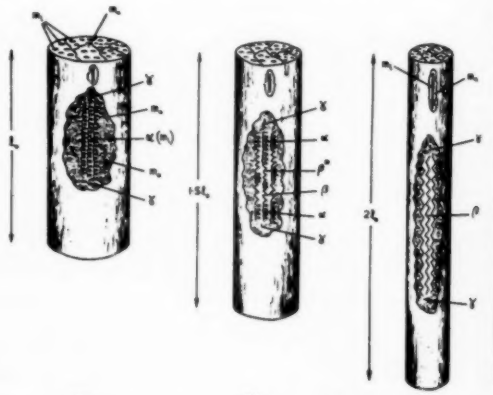
WITH the advent of rapid scanning X-ray diffractometry[1,2] it has become possible to investigate, more fully, certain aspects of crystallization in the fibrous proteins. In particular, it is now apparent that crystallization in wool keratin may be a fruitful field of study. One of the simplest systems to investigate is that of the keratin–water complex, both under external stress free, and tensile stress deformation, conditions. The degree of hydration of the keratin component is intimately related to the type of crystallization observed, and some aspects of this relationship are described here.

Present-day hypotheses[3,4] tend to support the view that unstrained wool keratin consists of 'crystalline' microfibrils embedded in an 'amorphous', or at least somewhat disordered, 'matrix'. Evidence from electron microscopy[5] suggests that the microfibril/matrix areal ratio is near unity, and data from infrared spectroscopy[6] are compatible with the idea that the most readily deuterium exchangeable fraction is an 'amorphous' region, and most probably the matrix.

The lateral dimensions of the microfibrils are approximately the minimum required[7] in order to secure a 'crystalline' X-ray reflexion. For this reason, and because the matrix is 'disordered', it is reasonable to ascribe the origin of the wide-angle X-ray diffraction pattern to interference from within the microfibrils. Before proceeding, it is necessary to distinguish between the configuration of a crystallographic component of structure and the configuration of the fibre as a whole. Thus, in the present case, the maximum amount of material available for the crystalline phase is 50 per cent, and it is normally assumed that an appreciable fraction of the molecules in the microfibrils are in the helical configuration which gives rise to the characteristic reflexions. However, we may also say, loosely, that the fibres are in the α-form, by which we simply mean that chains in the α-configuration are dominant in the crystalline regions.

Fig. 1*a* shows in outline the gross details of the generally accepted model of structure for wool keratin fibres in the 'dry' state. If the system is hydrated, for example, soaked in water at room temperature (18° C.) for a reasonable period, it is observed: (1) that the equatorial spacing about 9·8 Å. is little altered[8,9]; (2) (from the results of this investigation) that there is little change (less than 5 per cent) in the radially 'integrated' scattered intensity of the 9·8 Å. reflexion. These results and those of the deuterium exchange reaction[6] suggest that few water or deuterium oxide molecules penetrate into the helical interstices. Thus, under conditions of zero external stress the crystallographic configuration of both dry and hydrated keratin remains unaltered, at least to within the limitations of detectability of the present detecting system.

With the application of an external stress of sufficient magnitude to cause an appreciable strain, an entirely new and more complicated system is developed. The nature of the end-products of the reaction depend, in an as yet ill-defined manner, on



m_f, microfibrils; m_a, matrix; l_0, original length

Fig. 1. (*a*) Model of unstretched wool keratin. Microfibrils, α-transformable helices; γ, invariant fraction. Matrix, an initially disordered region of high water accessibility. (*b*) Wool stretched 50 per cent and dried. β-crystalline phase derived from α, β^*, a mixed α- and β-crystallite. Matrix, becoming strained and more ordered. (*c*) Wool stretched 100 per cent and dried. β-Crystalline phase derived from all the formerly ordered α-material. Matrix, attaining a further degree of order

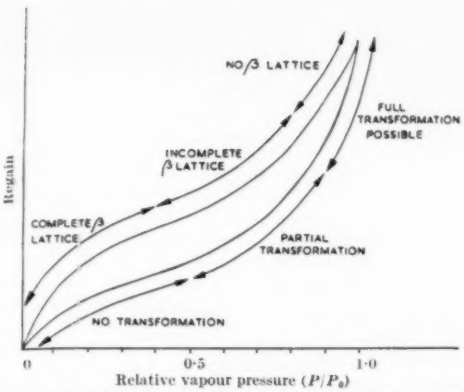


Fig. 2. The relationship of partial vapour pressure to the extent of crystallization in the β-form for wool keratin stretched up to 50 per cent. The absorption curve represents the extent of transformation of α-helices into β-crystallites, for stretching at the vapour pressures shown, after the fibres have been dried in the extended condition at 105° C. The desorption curve shows the extent of β-crystallization during lowering of the vapour pressure, after the fibres have been stretched wet to 50 per cent extension

the initial vapour pressure under which the fibres are stretched, and on the external conditions into which the fibres are finally brought.

In this connexion it is of value to consider the reaction products which may be obtained under two important limiting sets of conditions: (1) when a stress is applied to the fibres under a number of different partial water vapour pressures, and the then extended fibres are dried at 105° C. (conditions where there is a maximum likelihood of β-crystallites being formed); (2) when fibres are stretched wet and then the extent of formation of β-crystallites for progressively reduced vapour pressures is observed. In all cases the fibres were extended, either up to the maximum possible (determined by their initial conditions), or to an arbitrary and convenient limit of 50 per cent. The extent of crystallization was determined by a technique, depending on the magnitude of the radially integrated reflexion intensities, which has been reported elsewhere[10].

The results of one such set of crystallization experiments are summarized in Fig. 2. It will be seen that there is a strong correlation between the increase in the initial moisture content (regain) and the predisposition of the system to form β-crystallites after stretching and drying. Thus, it seems likely that an intermediate hydrate is a necessary precursor to an observable crystalline β-phase. The upper curve of Fig. 2, the desorption curve, confirms this view, for it is found that crystallization does not in fact occur until after the relative water vapour pressure falls below a certain value (0·8), and thereafter continues to increase until a limiting value, which represents the optimum equilibrium concentration of material in the ordered crystalline β-form, is attained.

In these experiments it was not found possible to obtain any evidence for the existence of crystalline β-keratin unless the vapour pressure of the extended material was reduced below that at which the extension took place. This result is in conflict with the interpretation of some observations reported earlier by Bendit[11].

The crystallization at 50 per cent extension of the mixed system of α- and β-keratin affords a striking illustration of the effectiveness of the rapid scanning technique, and some sequences from the X-ray diffractometer records are, therefore, reproduced in Fig. 3. These show the reappearance of the crystalline α- and β-reflexions as the solvent vapour pressure is reduced and indicate clearly the nature of the phenomenon taking place.

In earlier work[12] it has been established that at intermediate extensions the dehydrated equilibrium crystalline configuration contains a mixed crystallite which does not contribute to either the α- or the β-crystalline X-ray diffraction pattern. This has been designated β* (ref. 13). However, the evidence available now suggests that hydrated and extended material may consist of unchanged α-chains, hydrated α-chains, hydrated β*-material, and hydrated β-material. Further, it is observed[14] that the limiting reversible extension of the system is near 100 per cent. However, unconstrained α-helices would be expected to extend by at least 120 per cent[14], and measurements on the meridional reflexions confirm this[15]. Any interpretation of the changes in the equilibrium relative intensities of the α- and β-crystalline reflexions indicates that a smooth and continuous transition from the crystalline α- to β-phase occurs. These facts, taken together, suggest that there is an inextensible component of the microfibril in series with the helical α-crystalline phase. The work of Middlebrook[16] has suggested that cystine-rich regions alternate with cystine-poor regions along the fibre

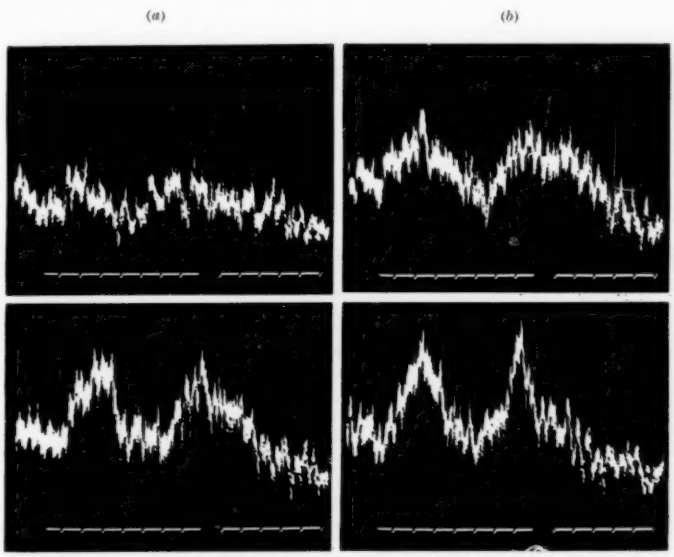


Fig. 3. Recrystallization of the wool keratin mixed crystal system at 50 per cent extension showing the reappearance of the equatorial $\alpha+\beta$ (9·8 Å.) and β (4·65 Å.) peaks as the solvent is progressively removed

axis and a component of higher sulphur content, γ-keratose[17], has been isolated. Thus, it is proposed to label the inextensible phase γ-keratose. Since this phase is not sufficiently well ordered to contribute to the discrete X-ray reflexions and is likely to be closely linked, it is suggested that hydration will have little effect on it. Because of this, it will be taken to form a largely unalterable component of the microfibrils. This analysis also leads to the value of 40 per cent for the 'crystallinity' of the fibre as a whole, a figure which compares well with those obtained by other workers and in particular 44 per cent by Hailwood and Horrobin[16].

Earlier interpretations[15] also suggest that it is only at extensions near 100 per cent that all the α-crystalline phase is transformed into the β-crystalline phase.

Thus the two extensions of 0 and 100 per cent form limiting cases for relatively simple keratin–water complexes. At any intermediate extension the possibility of several more components being present suggests itself. Table 1, therefore, indicates in outline the sequence of phases that may be involved in the α–β transformation, and underlines, too, the complexity of the situation in what was once thought[19] to be a simple polymorphic transformation. Next it is desirable to return to Fig. 1 and to contemplate the nature of the interaction between the matrix and the microfibrils.

Table 1. Sequence of Phases involved in the α–β Transformation in Wool Keratin

Condition of fibres	Phase states		Phase designation
	Microfibrils	Matrix	
Dry under no external stress	$\alpha + \gamma$	Dehydrated	α
Wet under no external stress	$\alpha + \gamma$	Hydrated	α_H
Wet under stress extended 50 per cent	$\alpha + \alpha_H + \beta^*_H + \beta_H + \gamma$	Hydrated	Mixed M_H hydrate
Dry under stress extended 50 per cent	$\alpha + \beta^* + \beta + \gamma$	Dehydrated	Mixed dehydrate M
Wet under stress extended 100 per cent	$\beta_H + \gamma$	Hydrated	β_H
Dry under stress extended 100 per cent	$\beta + \gamma$	Dehydrated	β

Since the transformation from crystalline α to crystalline β material is uniform, there is no evidence to suggest that marked gross crystallographic differences exist along the transformable microfibrillar component. On the other hand, evidence from the analysis of the stress-strain curves[14,19] leads us to expect three distinct phases which may affect the extensometric characteristics. Successively, the fibre becomes more difficult to transform, but since initially Young's modulus of the matrix may be supposed to be small[3], the hindering of the transformation by the matrix is also initially small.

It is, however, reasonable to suppose that on extension the initially disordered matrix becomes more ordered and also contributes increasingly to the resistance to deformation, while the stress contribution of the crystalline material follows a uniform course depending on the difference in energy between the α- and β-forms.

The recent work of Crewther and Dowling[20] has reaffirmed that there exists a high degree of interaction between the microfibrils and the matrix under suitable conditions, but there does not seem to be any direct evidence to suggest, as has Feughelman[21], that the microfibrils themselves have a periodic sequence of X (more easily extensible) and Y (less easily extensible) zones. All that can be definitely asserted, in the absence of the crystallographic transformation data, is that the system (microfibril–matrix), acting as a whole, produces the observed supercontraction and stress-strain discontinuities. The view to be taken here, however, is that as the crystallographic transformation in the microfibrils is reasonably uniform throughout the entire range of extensions, it is the matrix, with differing degrees of deformability, that is the source of complexities in the other observable physical parameters. The contribution of the microfibrils to the developed stress over the whole transformation-range may thus be derived from an extrapolation of the wet stress at low extensions, assuming the simple uniform transformation of the crystallites. On the basis of this assumption, it is possible to calculate a value for the difference in free energy between the α-phase and the β_H-phase using the relationship $\Delta F^0 = \lambda \frac{ARf}{k}$ (ref. 14). This equation holds for the condition [α] = [β], which is obtained near 50 per cent extension.

If we assume a single NH . . . O = C hydrogen bond in a Pauling and Corey type α-helix[22] to be the fundamental flow unit, we find, for hexagonal packing, the following values for certain of the required parameters:

A, the cross-sectional area $\simeq (5{\cdot}8)^2 \times 3{\cdot}46 \times 3{\cdot}7^{-1} \times 10^{-16}$ cm.^{2}; λ, the translation distance of a single segment of chain, $\simeq 6{\cdot}35 \times 10^{-8}$ cm.; $R = 1{\cdot}99$ calories/mole deg.; $k = 1{\cdot}375 \times 10^{-16}$ erg/deg.

The remaining parameter, f, the equilibrium stress, is rather difficult to establish accurately, owing to long-term stress relaxation. However, it might reasonably be argued that in so far as the reversible crystallographic transformation is concerned, equilibrium is, in fact, reached in a relatively short time (within the duration of the experiment). Stress relaxation continuing after this time may be due to a significant change in keratin structure not within the purview of present considerations. Such possibilities have been confirmed recently by Woods[14] and Kubu *et al.*, who have shown that the first (rapid) stage of relaxation involves hydrogen bonds only, while long-term relaxation depends to a large extent on primary (particularly disulphide) bond fission. If Crewther and Dowling's ideas[20] are substantiated, the matrix is, in fact, rich in sulphur in comparison with the transforming section of the microfibrils. Their suggestions thus provide confirmatory evidence for the keratin model outlined above. As an approximation, the extrapolated value of stress adopted by Peters and Woods[14] from Woods's earlier data may be chosen. This yields an extrapolated stress of $\simeq 8 \times 10^8$ dynes/cm.^{2} at $\simeq 50$ per cent extension in water at about 30° C. Since the microfibrils are in effect taking all the stress at low extensions, the effective loading is approximately twice this value, namely, 16×10^8 dynes/cm.^{2}.

This leads to a free-energy difference of $\simeq 4{\cdot}5$ kcal./mole, which may be compared with a figure derived by Ruoff *et al.*[23] of 4·7 kcal./mole, and thus further confirms the adequacy of the molecular model involved.

Further work on elucidating the transformation mechanism, and the factors involved in crystallization in wool keratin is proceeding.

I am grateful for the assistance of Prof. J. B. Speakman, H. J. Woods and the Wool Textile Research Council.

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PEAK-SHIFT TECHNIQUE IN GAS-LIQUID CHROMATOGRAPHY: TRIMETHYLSILYL ETHER DERIVATIVES OF ALCOHOLS

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STABLE solid crystalline derivatives with melting points in the range of 50°–300° C. have been of greatest interest for classical qualitative organic chemical analysis and identification. Recently, the growth of the analytical application of mass spectrometry[1,2] and particularly gas chromatography[3–11] has stimulated considerably greater interest in liquid derivatives of organic compounds with their increased volatility, reduced adsorption on solid surfaces and enhanced separation factors. Despite this, with the exception of several peak subtraction techniques and the hydrogenation of unsaturated materials[12], identification and analysis via the 'peak shift' resulting from conversion of volatile materials to volatile derivatives has received only limited attention. Some advantages, as well as complications, can be illustrated by consideration of a hydrogenated Fischer–Tropsch fraction, b.p. 110°–115° C., after hydrogenation obtained from a synthesis using iron nitride catalyst[13].

The principal components of the Fischer–Tropsch fraction were octane, 2-methyloctane, nonane, 1-butanol, 2-methylbutanol, and 1-pentanol. Treatment of the mixture with hexamethyldisilazane was used to convert the alcohols to their trimethylsilyl derivative[2]. Some retention volume data for three liquid phases are shown in Table 1. The trimethylsilyl ethers will emerge from the column before the parent alcohols on the polar polyesteramide 'Flexol 8*N*8' liquid phase (because of hydrogen bonding, the alcohols tend to be retained) and after the parent alcohols on Dow Corning 'Silicone 550' (solubility increased as solubility parameters are more nearly equal[14]). Thus, peak shift should be a definite aid to identification after treatment with a selective reagent such as hexamethyldisilazane. Furthermore, the volatile derivative provides access to another gas chromatographic parameter for identification similar to that obtained from chromatography of one compound on two liquid phases[15,16]. That is, a plot of log retention volume of parent compound versus log retention volume of derivative is linear for a homologous series. A plot of such values for Dow Corning 'Silicone 550' is shown in Fig. 1. The 2-methyl and normal alcohols apparently fall on different lines. The linearity of plots such as that shown in Fig. 1 is to be expected when the logarithm of retention volumes of both the parent compounds and derivatives are linear functions of the number of carbon atoms in the chain.

Difficulties are encountered when members of different classes occur in the same mixture[16] except when there is a large disparity in amounts present. Thus, the retention volume of 1-butyl trimethylsilyl ether coincides with that of octane on silicone oil and on 'Flexol 8*N*8', while the trimethylsilyl derivative of pentanol tends to emerge close to nonane. However, the disappearance of the alcohol peak is definite and the increase in the hydrocarbon peak, if there is a hydrocarbon present, is often apparent. The parent compound of interest may also be trapped and reacted with hexamethyldisilazane for definite identification. Other workers[10] at the U.S. Bureau of Mines have performed identifications similar to this by comparing retention volumes of sulphur-

Table 1. CORRECTED RETENTION VOLUMES* ON VARIOUS LIQUID PHASES AT 97·5°

	D.C. 'Silicone 550'		'Flexol 8*N*8'†		Pentatriacontane ‖	
Octane	68·8		108·0		184·8	
2-Methyloctane	—		—		351·0	
Nonane	140 §		227·0 §		402 §	
2-Methyl-1-propanol	27·6	48‡	123		—	
2-Methyl-1-butanol	63·0	100·4‡	—		—	210‡
2-Methyl-1-pentanol	128·8	180·5‡	—		—	
1-Propanol	17·8	35·6‡	72·4	64·7‡	—	65·8‡
1-Butanol	38·3	68·5‡	162	108·5‡	—	133‡
1-Pentanol	80·6	135‡	352	216·3‡	—	280‡
1-Hexanol	167	270·3‡	—	—	—	—
1-Heptanol	338	550‡	—	—	—	—

* In ml./gm. at column temperature (V_g of air = 0) and atmospheric pressure.
† Product of Union Carbide Chemical Co., 2,2′(2-ethylhexamido)-diethyl-di(2-ethylhexoate).
‡ For trimethylsilyl ether of indicated alcohol.
§ Calculated from other hydrocarbon retention data.
‖ From hydrogenation of 17-pentatriacontene (Armour and Co., Chicago, Illinois).

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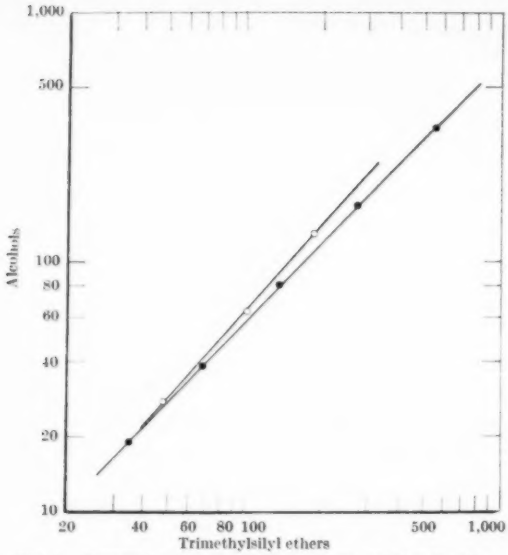

Fig. 1. Corrected retention volumes, ml./gm. on Dow Corning 'Silicone 550' at 97·5° C. Alcohols versus their trimethylsilyl derivatives (log log scale)
●, *n*-alcohols; ○, 2-methyl alcohols

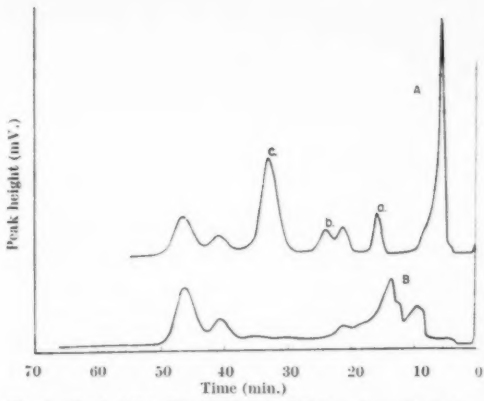

Fig. 2. 'Peak shift' after treatment of Fischer–Tropsch product with hexamethyldisilazane. *a*, Trimethylsilyl ether of 1-butanol; *b*, trimethylsilyl ether of 2-methyl-1-butanol; *c*, trimethylsilyl ether of 1-pentanol. B.P., 110–115° C.; flow, 40 c.c./min.; *λP*, 415 nm.; pentatriacontane column; *T*, 97·8° C.

containing materials and derived hydrocarbons after desulphurization.

Chromatograms illustrating the behaviour of the Fischer–Tropsch fraction on pentatriacontane before and after treatment with hexamethyldisilazane are shown in Fig. 2. From curve B, it is seen that the alcohols are not resolved and are eluted early (9–16 min.) from the non-polar hydrocarbon liquid phase so that the hydrocarbon portion of the chromatogram is simplified. The shifting of the alcohols after treatment with hexamethyldisilazane, as shown in curve *A*, is in itself diagnostic; in addition to identification, analysis of the alcohols as well as hydrocarbons is now possible on the non-polar liquid phase by calibration against one of the hydrocarbons or addition of an 'internal standard' which may be a hydrocarbon or a hydroxyl-containing material. The peak at 6–9 min. is due to excess hexamethyldisilazane and some hexamethyldisiloxane due to reaction with water[6]. The reaction with, and removal of, water by hexamethyldisilazane to form hexamethyldisiloxane is an additional advantage of this reagent. Peaks at about 21, 41, and 45 min. are due to octane, 2-methyloctane and nonane. Identity of the alcohols is indicated in Fig. 2.

Experience with the trimethylsilyl ethers as derivatives for alcohols indicates that additional development of convenient reagents for functional groups which react quantitatively to give volatile derivatives should increase the utility of gas chromatography for organic analysis appreciably. It is desirable also that such reagents be specific and that the retention volumes of derivatives obtained be considerably different from those of present compounds.

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MANNOSYL D-GLUCOSAMINE HYDROCHLORIDE ISOMERS

N-Mannosyl D-Glucosamine Hydrochloride

WHEN a D-glucosamine hydrochloride-D-mannose mixture (each 5 per cent w/v in water) was applied to Whatman No. 3 paper, heated for 15 min. at 100° and thereafter submitted to ionophoresis in 0·2 *M* acetate buffer *p*H 5 at 8 V./cm. for 4 hr. a new component, now identified as *N*-mannosyl D-glucosamine hydrochloride, was detected. This novel compound ($M_{\text{glucosamine}}$ 0·56) gave positive reactions with ninhydrin[1], the Elson–Morgan reagents[2], aniline hydrogen phthalate[3], alkaline silver nitrate[4] and alkaline triphenyltetrazolium chloride[4], but not with naphthoresorcinol[2]. In order to isolate

larger quantities of *N*-mannosyl D-glucosamine hydrochloride it was necessary to carry out the reaction described above using the sugar mixture (50 gm.) freeze-dried on to cellulose powder (200 gm.). Batches of the products (4–6 gm.) were then eluted with water, freeze-dried, dissolved in 0·3 *N* hydrochloric acid and applied to a column of 'Zeokarb 225' [H^+] (length, 53 cm.; diam., 8 cm.). Elution with 0·3 *N* hydrochloric acid and analysis of the 50 ml. fractions collected showed separations between D-mannose (tubes 20–30; optically active), 5-hydroxymethyl 2-furaldehyde[5] (tubes 86–96; absorption at 284 mμ), *N*-mannosyl D-glucosamine hydrochloride (tubes 96–106; determined by Elson–Morgan[6] and ninhydrin[7] reagents) and unreacted D-glucosamine (tubes 140–175; optically active, Elson–Morgan and ninhydrin-positive). The 5-hydroxymethyl 2-furaldehyde was characterized by its ultra-violet absorption spectrum ($\lambda_{max.}$ 284 mμ), chromatographic behaviour, partition coefficients between two different solvent systems (0·3 *N* hydrochloric acid–chloroform and 0·3 *N* hydrochloric acid–ether) and isolation of its 2 : 4-dinitrophenylhydrazone (m.p. and mixed m.p. 200–1°). The solution containing *N*-mannosyl D-glucosamine was neutralized by extraction with a chloroform solution of di-*n*-octylmethylamine[8] and freeze-dried (yield 50–100 mgm.).

N-mannosyl D-glucosamine hydrochloride showed no ultra-violet absorption and was virtually unchanged in 0·3 *N* hydrochloric acid at room temperature during two weeks. Complete hydrolysis of *N*-mannosyl D-glucosamine hydrochloride (300 mgm.) occurred when it was heated in water (30 ml.) at 100° for 12 hr. and was accompanied by extensive browning. The products were separated on the ion-exchange column and characterized as D-mannose, 40 mgm. (isolated as its *p*-nitroanilide m.p. and mixed m.p. 208°), a neutral sugar present in much smaller amount (separated from mannose by paper chromatography; probably glucose from its R_F value and the ionophoretic mobility of its borate complex), 5-hydroxymethyl 2-furaldehyde (isolated as its 2 : 4-dinitrophenylhydrazone m.p. 201°) and D-glucosamine hydrochloride (88 mgm. obtained crystalline with $[\alpha]_D$ + 86° (init.) → + 69° (equil.) (*c*, 1·69 in water) and further characterized as its *N*-carbobenzoxy derivative, m.p. and mixed m.p. 211°–213° (*d*)). When *N*-mannosyl D-glucosamine hydrochloride was left in sterile water at room temperature it was almost completely hydrolysed in 30 days to glucosamine hydrochloride and mannose. No ultra-violet absorption attributable to 5-hydroxymethyl 2-furaldehyde was detectable. Direct determination of the mannose (assayed with orcinol–sulphuric acid[9]; chromogen identical with that of authentic mannose) and glucosamine hydrochloride (Elson–Morgan reagents[6]; chromogen $\lambda_{max.}$ 530 mμ) content of *N*-mannosyl D-glucosamine hydrochloride revealed a ratio by weight of glucosamine hydrochloride to mannose of 98 : 95, confirming that it contained equimolar proportions of the two sugars. The instability of the new compound in aqueous solution precludes the possibility of an *O*-glycosyl linkage. When the condensation was effected between ^{14}C-mannose and glucosamine hydrochloride it was found that the 5-hydroxymethyl 2-furaldehyde obtained both from the reaction mixture and the degradation of the *N*-mannosyl-D-glucosamine was radioactive as indeed was the *N*-mannosyl D-glucosamine hydrochloride. Hence the 5-hydroxymethyl 2-furaldehyde originates from the mannose moiety as would be expected for a glycosylamine. *N*-mannosyl D-glucosamine hydrochloride gave negative results in the methylene blue, 2 : 6-dichlorophenol–indophenol and Seliwanoff tests for Amadori compounds[10,11], and its infra-red spectrum did not contain a peak at 1,720 $cm.^{-1}$ due to a carbonyl group such as has been observed in the infra-red spectra of certain Amadori compounds[12].

Indirect evidence in support of the glycosylamine structure accrues from a study of the formation of compounds analogous to *N*-mannosyl D-glucosamine hydrochloride by heating several other mixtures of amino-sugars with reducing sugars and their derivatives. Since no analogous compound was produced from *N*-acetylglucosamine and a reducing sugar or from glucosamine hydrochloride and non-reducing sugars (mannitol, α-methylmannoside, 1-deoxy D-glucose, sorbitol, dulcitol and α-methylglucoside) it appeared that such reactions were a consequence of reaction between the reducing group of a sugar (aldose, not a ketose) and the amino group of a second compound. Thus mannose formed a condensation compound not only with D-glucosamine and D-galactosamine hydrochlorides but also with the hydrochlorides of D-glucosaminol and 2-aminoglycerol. Another necessary condition appears to be that the C_3 position of the reducing sugar should not be substituted. Thus while condensation compounds were formed from 3-*O*-methyl and 3 : 4-di-*O*-methyl D-glucose and 3 : 4-di-*O*-methyl D-mannose when heated with D-glucosamine hydrochloride, no such compounds were detected with 2 : 3-di-, 2 : 3 : 6-tri- and 2 : 3 : 4 : 6-tetra-*O*-methyl D-glucose. Steric hindrance is the most likely cause of this phenomenon.

One of us (K. M.) is grateful to Glaxo, Ltd., for the award of a scholarship.

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6-*O*-Mannosyl D-Glucosamine Hydrochloride

Since the work reported in the previous article, we have found that under more vigorous conditions (3 hr. at 120°) disaccharides of the *O*-glycosyl type are also formed. The isolation and characterization of one of these, 6-*O*-mannosyl D-glucosamine hydrochloride, is the subject of the present communication.

After heat treatment, the resultant products were extracted with water and boiled for 12 hr. to hydrolyse the *N*-mannosyl D-glucosamine hydrochloride present since this had a similar elution position to the oxygen-linked disaccharides on 'Zeokarb 225' (H^+, 200–400 mesh, 4½ per cent cross-linking). Subsequent fractionation on the resin yielded the residual disaccharides in two major fractions *A* (250 mgm.) and *B* (100 mgm.), being the products from 10 gm. of reaction mixture. *B* was homogeneous ($R_{glucosamine}$ 0·35) on a paper chromatogram irrigated with the organic phase of butan-1-ol, ethanol and water (4 : 1 : 5) while *A* contained two components ($R_{glucosamine}$ 0·35 and 0·47). *B* reacted with ninhydrin[1], the Elson–Morgan reagents[2], alkaline silver nitrate[4], aniline hydrogen phthalate[3], alkaline triphenyltetrazolium chloride[4], but not naphthoresorcinol[3]. It had $[\alpha]_D^{18}$ + 55° (*c*, 1·0 in water) and a mobility 0·7 that of glucosamine when separated by paper electrophoresis in 0·2 *M* acetate buffer *p*H 5.

XUM

Complete hydrolysis of *B* with 2 *N* hydrochloric acid for 3 hr. at 100° yielded equimolar amounts of mannose and glucosamine hydrochloride. These results suggested that *B* was an *O*-mannosyl glucosamine hydrochloride. *N*-acetylation[12] of *B* (250 mgm.) yielded a product (250 mgm.; $R_{glucosamine}$ 0·6) the chromogen of which exhibited 26 per cent of the absorption at 590 mμ of an equal weight of *N*-acetylglucosamine in the Morgan–Elson determination[14]. Reduction of *N*-acetyl *B* (200 mgm.) with sodium borohydride (200 mgm.) in water (20 ml.) at room temperature for 5 hr. yielded a crystalline *O*-mannosyl 2-acetamido 2-deoxy glucitol (43 mgm.; from methanol–acetone), m.p. 202°–203°; $[\alpha]_D$ − 50·6° (*c*, 1·0 in water). Found: C, 43·5; H, 6·9; N, 3·6. Calc. for $C_{14}H_{27}O_{11}N$: C, 43·6; H, 7·1; N, 3·6 per cent. Hydrolysis with 2 *N* hydrochloric acid for 3 hr. at 100° gave D-mannose and D-glucosaminol, establishing that the glucosamine-moiety formed the reducing portion of the original disaccharide. On periodate oxidation, the *O*-mannosyl 2-acetamido 2-deoxy glucitol consumed 3·9 moles of periodate and liberated 1·8 moles of formic acid per mole of alcohol. No formaldehyde was liberated. Reduction of the periodate oxidized disaccharide alcohol followed by hydrolysis with 0·5 *N* hydrochloric acid at 100° for 1 hr. yielded *inter alia* 2-amino 2-deoxy glyceritol. Hence the disaccharide alcohol was 6-*O*-D-mannopyranosyl 2-acetamido 2-deoxy D-glucitol. Application of Hudson's rules in the manner used by Stavely and Fried[15] to assess the configuration of the mannosyl residue in mannosido-streptomycin gives a value (*A*) of *c.* − 18,000 for the contribution of carbon atom one of the mannose residue to the molecular rotation. The negative value of *A* (cf. A_{OMe} = 14,230) indicates a β-linkage in the disaccharide alcohol. The rotation of the 6-*O*-mannosyl glucosamine hydrochloride, that is, + 55°, compares favourably with the rotations of related 6-*O*-β-D-mannosyl disaccharide derivatives[16].

The disaccharide probably arises from a condensation between 1 : 6-anhydro-D-mannopyranose, formed during the pyrolysis, and D-glucosamine hydrochloride. Indeed, a component with the same R_F value as the β-anhydro sugar has been detected in the pyrolysis mixture, which is not unexpected since pyrolysis is used in the preparation of the anhydro sugar from ivory nut mannan[17]. It is noteworthy that a heated mixture (1 : 1) of this anhydro sugar and D-glucosamine hydrochloride gave a 20 per cent greater yield (by weight and by Elson–Morgan reaction[6]) of *O*-mannosyl glucosamine type disaccharides than a heated D-mannose D-glucosamine hydrochloride mixture. Furthermore, L-rhamnose (6-deoxy-L-mannose), which cannot form a 1.6-anhydro derivative, did not yield Elson–Morgan-positive[6] disaccharides.

We are indebted to Dr. N. K. Richtmyer for a sample of 1 : 6-anhydro β-D-mannopyranose and one of us (D. B. E. S.) thanks the University of Birmingham for a scholarship.

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COUPLING OF PHOSPHORYLATION TO ELECTRON AND HYDROGEN TRANSFER BY A CHEMI-OSMOTIC TYPE OF MECHANISM

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At present, the orthodox view of the coupling of phosphorylation to electron and hydrogen transfer in oxidative and photosynthetic phosphorylation stems from knowledge of substrate-level phosphorylation[1,2]. It is based, consequently, on the idea that water is expelled spontaneously between two chemical groups, *A* and *B*, by the formation of a strong bond (of low hydrolysis energy), and that subsequent or simultaneous oxidation or reduction of *A*–*B* to *A*–*B** can result in a weakening of the bond, popularly written $A \sim B^*$, so that adenosine triphosphate (ATP) can be synthesized by coupling the opening of $A \sim B^*$ to the closing of the 'high-energy bond' between adenosine diphosphate (ADP) and phosphorus through group transfer systems of appropriate substrate and oxido-reduction-carrier specificities[3–5]. There are a number of facts about the systems catalysing oxidative and photosynthetic phosphorylation that are generally acknowledged to be difficult to reconcile with this orthodox (chemical) view of the mechanism of coupling. For example: (*a*) The hypothetical 'high-energy' intermediates (for example, reduced diphosphopyridine nucleotide (DPNH) ~ ?, reduced flavin adenine

dinucleotide (FADH) ~ ?, cytochrome ~ ?) are elusive to identification[4,6]. (*b*) It is not clear why phosphorylation should be so closely associated with membranous structures[7–9]. It has sometimes been assumed that digitonin-treated mitochondrial 'particles' can couple oxidation to phosphorylation without membranes[10]; but it is doubtful whether this assumption can be justified, for such 'particles' give only poor respiratory control and contain much lipid[11], which may well exist as a leaky membrane[12]. (*c*) Coupling may vary with the stress, causing variation of respiratory control without corresponding variation in phosphorus/oxygen quotient[4,13]—a phenomenon difficult to explain in terms of molecular stoichiometry. (*d*) Hydrolysis of external ATP by mitochondria causes reduction of internal DPN, accentuated by the oxidation of succinate[14]. There is disagreement as to what complex assumptions offer the better explanation[15]. (*e*) Uncoupling can be caused at all three hypothetical oxido-reduction sites in mitochondria by agents that do not share an identifiable specific chemical characteristic[4] (for example, dinitrophenol, dicoumarol, salicylate, azide). (*f*) Unexplained swelling and shrinkage phenomena accompany the activity of the phosphorylation systems[7,9e,11,16].

Structural features have been invoked as the causes of the departure of the phosphorylation systems from 'ideal' behaviour[4,14,17]; but, as Green has pointed out in a far-sighted paper[18], although the structural features have been recognized as playing an important part in the catalytic activity of multi-enzyme systems, they have so far been treated rather conservatively. The structural (or supramolecular) features have, in fact, generally been regarded only as modifiers of the basic chemical type of coupling process outlined above. The general conception of enzyme-catalysed group translocation that we have been developing in my laboratory for some years[19] offers a more radical approach to the problem; but this has not, so far, been made use of by those working in the field of oxidative and photosynthetic phosphorylation.

The purpose of this article is to suggest that in view of the difficulties confronting the orthodox chemical conception of coupling in oxidative and photosynthetic phosphorylation, one might now profitably consider the basic requirements and potentialities of a type of mechanism that is based directly on the group translocation conception. This type of mechanism differs fundamentally from the orthodox one in that it depends absolutely on a supramolecular organization of the enzyme systems concerned. Such supramolecularly organized systems can exhibit what I have called chemi-osmotic coupling[19c,20], because the driving force on a given chemical reaction can be due to the spatially directed channelling of the diffusion of a chemical component or group along a pathway specified in space by the physical organization of the system[20]. We shall consider chemi-osmotic coupling between the so-called ATPases on one hand and the electron and hydrogen transfer chain on the other: mediated by the translocation of electrons and the elements of water across the membrane of mitochondria, chloroplast grana, and chromatophores.

The first basic feature of the chemiosmotic coupling conception is a membrane-located reversible 'ATPase' system[20b] shown very diagrammatically in Fig. 1. This system, which may include lipids and other components as well as proteins, is assumed to be anisotropic so that the active centre region (indicated by the dotted circle) is accessible to OH′ ions but not H^+ ions from the left (inside organelle), and to H^+ ions but not OH′ ions from the right (cytoplasm side of organelle). The active centre, like that of phosphokinases in general, is assumed to be relatively inaccessible to water as water. To illustrate the hydrolytic activity of the system, the OH′ ion is depicted as diffusing from left to right and finally combining at the active centre region with the terminal phosphorylium (P^+) derived from the ATP (ADP—O′—P^+) giving inorganic phosphate (POH) and ADP having a terminal ionized oxygen (ADP—O′), assuming the right-hand phase to be at about *p*H 7 and at electrical neutrality. The elegant conception of the transfer of phosphoryl as phosphorylium ion is due to Lipmann[21]. The system, of course, catalyses hydrolysis equilibrium, and the reverse or phosphokinetic activity of the 'ATPase' is indicated by the single barbs on the arrows of Fig. 1. In the case of the phosphokinetic activity, the OH′ ion is depicted as passing down a free-energy gradient towards the left from an inorganic phosphate group (POH) that passes to the active centre region from the right. The phosphorylium ion (P^+), created by the withdrawal of the OH′, is attacked by the negative atom ($-R'$) in the active centre region, and the phosphorylium ion is then donated to the terminal oxygen of ADP—O′ to give ATP. The chemi-osmotic coupling hypothesis depends thermodynamically on the fact that in such an anisotropic ATPase system, the electrochemical activity of the water at the active centre ($[H_2O]_c$), which determines the poise of the hydrolysis equilibrium in the ATP/ADP system, would be given, not by the product of $[H^+] \times [OH']$ in the aqueous phases *L* or *R*, but by the product $[H^+]_R \times [OH']_L$ (where [] stands for electrochemical activity, and *R* and *L* for right- and left-hand phases respectively). The ratio of the electrochemical activity [ATP]/[ADP] (including all ionic forms) can be raised, consequently, and the ATPase activity can be reversed to give an ADP phosphokinase activity proportional to the lowering of $[H_2O]_c$, in accordance with the mass-action law for hydrolysis equilibrium, written to include the elements of water as follows:

$$\frac{[ATP]}{[ADP]} = \frac{[P]}{K_1[H_2O]_c} \qquad (1)$$

The electrochemical activity of a component in a certain place defines absolutely the escaping tendency of the particles of the component due both to the

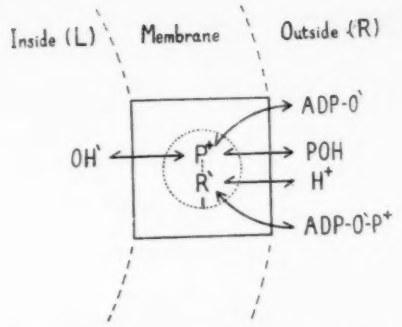

Fig. 1. Anisotropic reversible 'ATPase' system located in an ion-impermeable membrane between aqueous phases *L* and *R*

chemical and to the electrical pressure to which the particles are subject in that place at equilibrium. Since

$$K_2 = \frac{[OH']_L \times [H^+]_L}{[H_2O]_L} \quad (2)$$

and K_2 is independent of the medium because we are using electrochemical activities, we can describe the electrochemical activity of the water at the active centre of the 'ATPase' system as follows:

$$[H_2O]_c = [H_2O]_{aq.} \times \frac{[H^+]_R}{[H^+]_L} \quad (3)$$

where $[H_2O]_{aq.}$ stands for the electrochemical activity of water in the aqueous physiological media of phases L or R, and is equivalent to about 55·5 M water.

By the definition of the electrochemical activity:

$$\frac{[H^+]_L}{[H^+]_R} = 10^{\,pH_{R-L}} \times 10^{(mV._{L-R})\frac{F}{2303\,RT}} \quad (4)$$

where pH_{R-L} is the pH of phase R minus that of phase L; $mV._{L-R}$ is the membrane potential in millivolts, positive in phase L; R is the gas constant; F is the faraday; and the factor $\frac{F}{2303\,RT}$ is approximately 1/60. It can be seen from equation (4) that the ratio $[H^+]_L/[H^+]_R$ is multiplied by a factor of 10 for each pH unit more negative on the left, relative to the right, and for each 60 mV. membrane potential, positive on the left. Equations 1 and 3 show that the ratio [ATP]/[ADP] at equilibrium is determined by $[H^+]_L/[H^+]_R$ as follows:

$$\frac{[ATP]}{[ADP]} = \frac{[P]}{K_1[H_2O]_{aq.}} \times \frac{[H^+]_L}{[H^+]_R} \quad (5a)$$

When the right-hand phase (representing the cytoplasm) is the region of the zero or reference potential, the electrochemical activity ratio of total ATP to ADP will be nearly the same as the corresponding concentration ratio, and [P] will correspond approximately to the inorganic phosphate concentration. Thermodynamic data[22] show that at pH 7 and at physiological temperatures the 'hydrolysis constant' as usually defined or the product $K_1[H_2O]_{aq.}$ is approximately 10^5; and when [P] is at the physiological level of 10^{-2} M, equation (5a) can be written:

$$\frac{[ATP]}{[ADP]} \simeq 10^{-7} \frac{[H^+]_L}{[H^+]_R} \quad (5b)$$

Thus, the [ATP]/[ADP] equilibrium can be poised centrally through the anisotropic 'ATPase' by making the ratio $[H^+]_L/[H^+]_R$ about 10^7. Equation 3 shows that this could be done, for example, by poising the left side 2 pH units below and 300 mV. above the right side. Such is the basic thermodynamic conception of the mechanism of reversal of the 'ATPase' activity. In kinetic terms, the reversal of the 'ATPase' activity can be understood by regarding the electrochemical activity gradient of hydrogen and hydroxyl ions across the active centre region of the 'ATPase' as the cause of the donation of the phosphorylium ion to the negative acceptor atom ($-R'$) by the simultaneous withdrawal of an OH′ ion from the inorganic phosphate down a steep gradient to the left and of an H^+ ion from $-RH$ to the right to ionize $-RH$ or to prevent H^+ from competing with phosphorylium ion for the acceptor $-R'$. Dehydration is accomplished by using the high $[H^+]$ region as a sink for OH′ and the high [OH′] region as a sink for H^+. It should be understood that the phosphorylation of ADP can be strictly described as dehydration only when the standard state is less than pH 6 (see Lipmann[21]). Since, however, the hydrogen atom of the terminal hydroxyl group of ADP, involved in phosphorylation, is dissociated reversibly as an H^+ ion, when the standard state is taken as pH 7, the phosphorylation process is mainly that of dehydroxylation. From the kinetic point of view, the fundamental processes involved are dehydroxylation + deprotonation, or dehydroxylation. It is relevant to note that phosphorylation is not directly caused by raising $[H^+]$, but is due to depression of [OH′]. For this reason, the hydrogen ion depicted in phase R of Fig. 1 (and correspondingly in Figs. 2 and 3) is shown as equilibrating with the active centre region of the 'ATPase' system, but not as being withdrawn stoichiometrically in relation to the withdrawal of OH′.

The second basic feature of the chemi-osmotic coupling conception is the electron and hydrogen translocation system, which is assumed to create the gradient of electrochemical activity of H^+ and OH′. Unlike the conception of the anisotropic 'ATPase', the idea of the anisotropic o/r system is not new, but stems from the work of Lund[23], and Stiehler and Flexner[24], and was first stated explicitly by Lundegårdh[25] more than 20 years ago. Lundegårdh's idea was more exactly defined in relation to ion transport by Davies and Ogston[26], and was elaborated by Conway[27]. It has been excellently reviewed by Robertson[28].

Fig. 2 illustrates how the electron transfer can affect the ratio $[H^+]_L/[H^+]_R$. The electron translocation and 'ATPase' systems are depicted as being placed in opposition in a charge-impermeable membrane. The hydrogen ions generated on the left and the hydroxyl ions generated on the right by the electron translocation system dehydrate ADP and inorganic phosphate (now simply denoted by P) to form ATP by withdrawing hydroxyl ions to the left and hydrogen ions to the right through specific translocation paths in the 'ATPase' system as described here. Conversely, of course, the effect of the back pressure of ATP hydrolysis is to force the o/r system towards reduction on the left (inside the

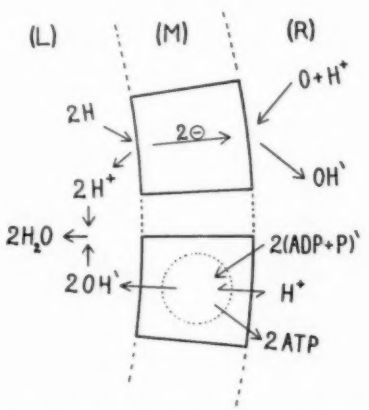

Fig. 2. Electron transport system (above) and reversible 'ATPase' system (below) chemiosmotically coupled in a charge-impermeable membrane (M) enclosing aqueous phase L in aqueous phase R

organelle). Note that the stoichiometry is 2 ATP per O, as in succinate oxidation by mitochondria. As shown in this simple diagram, the dehydrating force driving the phosphokinetic activity of the 'ATPase' system would be due largely to the chemical potential differential of the H^+ and OH' ions across the membrane, which would have to show as a pH difference of some 7 units across the membrane, acid on the left when the [ATP]/[ADP] equilibrium was poised centrally (see equations (5*b*) and (4)). We assume, however, that exchange diffusion carriers, as defined by Ussing[29], are present in the membrane and that they will allow strictly coupled one-to-one exchange of H^+ against K^+ or of OH' against Cl', for example. The pH differential would thus tend to be reduced to a relatively small figure and would be equivalently replaced by a membrane potential as described by equation (4).

When the oxido-reduction and phosphorylation systems are in chemi-osmotic equilibrium, one ATP molecule will be produced per electron translocated across the membrane. The relationship between the o/r potential span (ΔE) of the electron and hydrogen translocation system and the poise of the [ATP]/[ADP] equilibrium will be given as follows:

$$\frac{[ATP]}{[ADP]} = \frac{[P]}{K_1[H_2O]_{aq.}} \times 10^{\frac{\Delta E.F}{2303\,RT}} \qquad (6a)$$

or approximately:

$$\frac{[ATP]}{[ADP]} = \frac{[P]}{10^5} \times 10^{\Delta E/60} \qquad (6b)$$

ΔE being in millivolts. It should be understood that ΔE, as defined here, is equivalent to the free-energy change in the electron-translocating system per electron translocated. Assuming that ATP synthesis were occurring when the [ATP]/[ADP] ratio was poised centrally at pH 7 and in the presence of 10^{-2} M inorganic phosphate, the o/r span, ΔE, would have to be about 420 mV. This, of course, being the equilibrium potential, represents the minimum o/r span of the electron and hydrogen translocation system required to drive ATP synthesis under the conditions specified above. The span between the succinate–fumarate couple and oxygen at 76 mm. mercury pressure is about 750 mV.—well above the minimum ΔE of 420 mV.

In practice, the mitochondrial or chloroplast membrane across which the chemi-osmotic coupling may be organized would allow a certain amount of ion leakage, and the translocation paths for H^+ and OH', connecting the internal and external phases (L and R) with the active centre region of the 'ATPase', would not be expected to have absolute specificity for H^+ and OH' respectively. Consequently, equations (6*a*) and (6*b*) would represent the practical state of affairs in the most tightly coupled systems. On 'loosening' the membrane system, or if 'uncoupling' were effected by catalysing the equilibration of H^+ and charge across the lipid of the membrane with reagents like dinitrophenol or salicylate, the relationship between the poise of the [ATP]/[ADP] ratio and ΔE in the steady state would be described by the inequality:

$$\frac{[P]}{K_1[H_2O]_{aq.}} \leqslant \frac{[ATP]}{[ADP]} \leqslant \frac{[P]}{K_1[H_2O]_{aq.}} \times 10^{\frac{\Delta E.F}{2303\,RT}} \qquad (6c)$$

The outer terms of equation (6*c*) represent the extreme values of the [ATP]/[ADP] ratio from complete uncoupling on the left to complete coupling on the right.

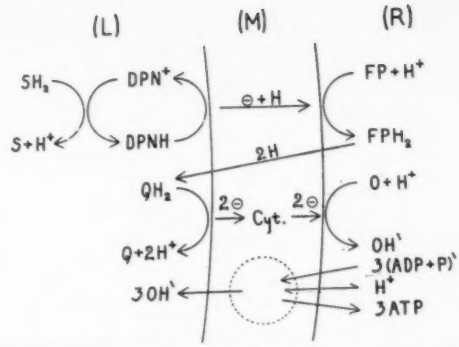

Fig. 3. Diagram of chemi-osmotic system for coupling phosphorylation to the oxidation of substrate (SH_2) through DPN, FP (tentatively identified with flavoprotein), Q (tentatively identified with a quinone) and the cytochromes (*Cyt.*). The other conventions are as in Figs. 1 and 2

Fig. 3 shows, in principle, a rather fuller description of oxidative phosphorylation, in which I have included the o/r components FP and Q, tentatively identified with flavoprotein and quinol–quinone systems respectively. The main aim is to illustrate how a stoichiometry of 3 ATP per O can readily be obtained for substrate (SH_2) oxidation through DPN, by the obligatory transport of one (net) hydrogen atom inwards per O, owing to the spatial arrangement of the electron and hydrogen transfer chain and the zero, one, or two hydrogen-transfer characteristics of the carriers involved. In this system, the span of both parts of the o/r chain across the membrane would have to be poised against the same ratio $[H^+]_L/[H^+]_R$ at equilibrium, according to equation (5*a*). Using the conventions of Dixon[2], the $-\Delta F$ value for the DPN/DPNH couple at about pH 5 would be some 3,000 cal. and the $-\Delta F$ for FP (corresponding to an E'_0 (pH 7) of -60 mV.) (ref. 30) would be about 17,000 cal., giving a span of 14,000 cal.; equivalent to a ΔE of 600 mV. Assuming the $-\Delta F$ of the Q system to be about 24,000 cal., corresponding to an E'_0 (pH 7) of + 100 mV., as in the ubiquinone system[31], the span from the Q system to oxygen at 76 mm. mercury pressure would be equivalent to a ΔE of about 750–100 = 650 mV. The tendency of the two o/r values to drift together and the exact magnitude of the composite o/r potential would, of course, depend on many factors that it would be premature to consider here. It will suffice to point out at present that the value of 600–650 mV. for ΔE is appropriately above the required minimum of 420 mV. and that the proposed system is thus in accord with the thermodynamic facts.

The above basic chemi-osmotic conception can be applied to photosynthetic phosphorylation with the difference that the electron and hydrogen translocation are seen as being driven, not by the affinity of oxygen for the hydrogen atoms and electrons, but by the energy of the absorbed photons, according to the type of mechanism described by Calvin[26]. It can readily be shown that in a chemi-osmotically coupled system for non-cyclic photophosphorylation, the photon-activated movement of 2 electrons and 2 hydrogen atoms outwards through the membrane of the grana would produce one O_2 and 2 ATP

molecules. Similarly, in non-cyclic photophosphorylation, the skew of $[H^-]_L/[H^-]_R$, and thus the synthesis of ATP, could be caused by the photon-activated passage of equal numbers of hydrogen atoms and electrons in opposite directions across the membrane of the grana or chromatophores.

The facts that were listed at the beginning of this article as being difficult to reconcile with the orthodox chemical conception of the mechanism of coupling phosphorylation to electron and hydrogen transfer can now be reconsidered in relation to the chemi-osmotic coupling hypothesis: (*a*) The elusive character of the 'energy-rich' intermediates of the orthodox chemical coupling hypothesis would be explained by the fact that these intermediates do not exist. (*b*) According to the chemi-osmotic coupling hypothesis, the differential of the electrochemical activity of the hydrogen and hydroxyl ions across the membrane, generated by electron transport, causes the specific translocation of hydroxyl and hydrogen ions from the active centre of the so-called ATPase system, thus effectively dehydrating ADP + P. The charge-impermeable membrane would therefore be an absolute requirement for tight coupling. (*c*) Coupling would be expected to vary with the extent of leakiness or strain in the membrane, determined, of course, by the osmotic and electrical stress. (*d*) The internal components of mitochondria such as DPN would tend to be reduced by the high electrochemical activity of H^+ caused by hydrolysis of external ATP, which would withdraw OH' ions from the inside in competition with the electron-transport system. This effect would be accentuated by oxidation of succinate (which would raise the internal value of $[H^+]$) but not, of course, by substrates such as acetoacetate that directly oxidize DPNH. (*e*) Uncoupling would be caused by lipid-soluble reagents, such as DNP, salicylate, azide, and ammonia, catalysing equilibration of H^+ or OH' and charge across the membrane. (*f*) According to the chemi-osmotic type of hypothesis, the coupling of phosphorylation to electron and hydrogen translocation would cause considerable electrical and mechanical stress in the membrane across which coupling was effected. Complex swelling and shrinkage effects would therefore be expected to accompany the activity of the system.

It is evident that the basic features of the chemi-osmotic coupling conception described here and elsewhere[20] are in accord with much of the circumstantial evidence at present available from studies of oxidative and photosynthetic phosphorylation. This simple hypothesis also has the merit that it represents the result of carrying to its logical conclusion the present trend towards recognizing the equivalent status of supramolecular and molecular features in the channelling of chemical processes in living organisms[18]. Further experimental support for the chemi-osmotic coupling conception may best be sought by attempting to characterize separately each of the three hypothetical basic elements of which the system is thought to be built: (1) the anisotropic 'ATPase' system which I have defined above; (2) the anisotropic *o/r* system of the type originally defined by Lundegårdh; (3) the specific charge-impermeable membrane in which the systems 1 and 2 are supposed to be orientated in opposition. Work along these three lines is proceeding in my laboratory.

In the exact sciences, cause and effect are no more than events linked in sequence. Biochemists now generally accept the idea that metabolism is the cause of membrane transport. The underlying thesis of the hypothesis put forward here is that if the processes that we call metabolism and transport represent events in a sequence, not only can metabolism be the cause of transport, but also transport can be the cause of metabolism. Thus, we might be inclined to recognize that transport and metabolism, as usually understood by biochemists, may be conceived advantageously as different aspects of one and the same process of vectorial metabolism[20a,32].

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TWO SEROLOGICALLY ACTIVE TRISACCHARIDES ISOLATED FROM HUMAN BLOOD-GROUP A SUBSTANCE

By I. A. F. LISTER CHEESE and Prof. W. T. J. MORGAN, C.B.E., F.R.S.

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IT is recognized that the serological characters of human blood-group specific mucopolysaccharides are due to relatively simple carbohydrate structures[1]. A direct means of studying these characters in detail consists in the examination of hydrolysis fragments which retain a measure of the serological activity of the intact substance.

Côté and Morgan[2] reported the isolation of five disaccharides from group *A* substance and showed that one of them, believed to be *O*-α-*N*-acetylgalactosaminoyl-(1 → 3)-galactose, was serologically active and inhibited the agglutination of *A* cells both by human *iso*-agglutinins and by an *A* specific reagent prepared from lima bean. They concluded that the disaccharide had a structure more closely associated with *A* specificity than any that had then been identified. This work has been extended and two trisaccharides showing *A* specificity have been isolated and are now described.

Blood-group *A* substance (22 gm.) was heated as a 2 per cent w/v solution in 0·1 *N* hydrochloric acid at 100° for 30 min. The solution was cooled and thoroughly dialysed to recover material of low molecular weight. This procedure was repeated on the indiffusible material, subsequent hydrolysis periods being 0·5 hr., 1 hr. and 2 hr. After removal of acid by passage through 'Deacidite *E*' (carbonate form) the diffusible material was broadly fractionated on a charcoal–'Celite' column into monosaccharides, disaccharides and higher oligosaccharides.

Higher oligosaccharides (1·5–2 gm.) were fractionated further on a charcoal–'Celite' column (70 cm. × 3·6 cm.) by gradient elution with aqueous ethanol (30 per cent v/v → 5 l.). Fractions of about 40 ml. were collected and examined by chromatography. All fractions were found to contain mixtures of sugars, and one group only (fractions 106–118) has so far been resolved. These fractions contained little of substances other than the two major trisaccharides now to be described. The material (230 mgm.) was fractionated by paper chromatography (solvent *A*, butanol–pyridine–water, 3 : 1 : 1, Whatman 3 *MM* paper) to yield trisaccharide I (28 mgm.) and trisaccharide II (40 mgm.). Further purification was carried out by eluting these materials from charcoal columns.

Trisaccharide I $[\alpha]_D$ + 147° was homogeneous by the criteria of paper chromatography ($R_{lactose}$: 0·97, in solvent *A*, Whatman 3 *MM* paper) and paper ionophoresis (M_G: 0·39, in borate buffer, *p*H 10). With benzidine-trichloroacetic acid[3] spray reagent it gave a yellow-brown colour similar to that given by *O*-β-galactosyl-(1 → 4)-*N*-acetylglucosamine. It reacted only feebly with the Ehrlich reagents of Partridge[4]. Complete acid hydrolysis yielded galactose, galactosamine and glucosamine, which were identified chromatographically. Determination of the sugars by Wilson's method[5] showed them to be present in equimolecular amounts. A preparation of enzyme from *Trichomonas foetus*[6] which destroyed the serological activity of *A* substance hydrolysed the trisaccharide to galactose and the two *N*-acetylated amino-sugars, but at an intermediate stage in the hydrolysis it was possible to detect a Morgan–Elson-negative saccharide indistinguishable chromatographically from *O*-β-galactosyl-(1 → 4)-*N*-acetylglucosamine, which had also been identified by Côté and Morgan[2] as a constituent unit in *A* substance.

Oxidation of the trisaccharide (0·06 m*M*) after reduction with sodium borohydride was carried out in 0·4 m*M* periodate, *p*H 3–5 at room temperature. Selective oxidation of the alcohol terminal takes place under these conditions[7]. The release of formaldehyde—1·00 mole per 590 gm. sugar—was complete within 10 min. A similar rapid and complete liberation of 1 mole proportion of formaldehyde was observed in the oxidation of reduced *O*-β-galactosyl-(1 → 4)-*N*-acetylglucosamine.

The trisaccharide was partially hydrolysed with *N* sulphuric acid at 100° for 36 min. and the hydrolysate examined by paper chromatography (solvent *A*). Among the products, which included the three constituent sugars and a little unchanged trisaccharide, there were found two reducing substances indistinguishable from *O*-α-*N*-acetylgalactosaminoyl-(1 → 3)-galactose and *O*-β-galactosyl-(1 → 4)-*N*-acetylglucosamine. It is concluded that trisaccharide I is most probably *O*-α-*N*-acetylgalactosaminoyl-(1 → 3)-*O*-β-galactosyl-(1 → 4)-*N*-acetylglucosamine.

In serological tests the trisaccharide was found to inhibit not only the agglutination of *A* cells by the common anti-*A* reagents, but also the lysis of sheep cells by rabbit anti-*A* immune serum and complement. *O*-α-*N*-acetylgalactosaminoyl-(1 → 3)-galactose alone of the disaccharides tested showed the same properties (Table 1).

Trisaccharide II $[\alpha]_D$ + 136° was homogeneous by chromatographic criteria ($R_{lactose}$: 1·10, in solvent *A*, Whatman 3 *MM* paper; M_G: 0·39, in borate buffer, *p*H 10). Both the benzidine-trichloroacetic acid spray reagent, with which it gave a dark

Table 1. Amounts of *A* Substance and Sugars giving Inhibition of (*a*) Human *Iso*-agglutinins and (*b*) Rabbit Anti-*A* Sheep Cell Hæmolysins

Substance	Minimum amount of substance giving inhibition (μgm./0·1 ml.)	
	a	*b*
Human *A* substance	0·04	0·01
N-acetylgalactosamine	> 1,000	> 1,000
α-Methyl-*N*-acetylgalactosaminide	500	500
O-α-*N*-acetylgalactosaminoyl-(1→3)-galactose	60	250
O-α-galactosyl-(1→3)-galactose	> 1,000	> 1,000
O-β-galactosyl-(1→3)-*N*-acetylglucosamine	> 1,000	> 1,000
O-β-galactosyl-(1→4)-*N*-acetylglucosamine	> 1,000	> 1,000
Trisaccharide I	60	15
Trisaccharide II	60	30

greenish-brown colour, and the Ehrlich reagents, provided ready means of detecting the substance. The component sugars, identified and determined as for trisaccharide I, were galactose, galactosamine and glucosamine in equimolecular amounts. Hydrolysis with the enzyme preparation obtained from *T. foetus* gave galactose and the two *N*-acetylated aminosugars.

Trisaccharide II, partially hydrolysed with *N* acid and examined as described for trisaccharide I, gave the same three constituent sugars and *O*-α-*N*-acetylgalactosaminoyl-(1 → 3)-galactose. In place of *O*-β-galactosyl-(1 → 4)-*N*-acetylglucosamine there was found a Morgan–Elson-positive spot corresponding to *O*-β-galactosyl-(1 → 3)-*N*-acetylglucosamine.

Treatment of the substance with 0·05 *N* sodium carbonate at 18–20° for 30 min. yielded, apart from some unchanged trisaccharide, several 'direct' Ehrlich chromogenic substances and a reducing substance indistinguishable in both chromatography and ionophoresis from *O*-α-*N*-acetylgalactosaminoyl-(1 → 3)-galactose. The lability of the trisaccharide to alkali and the high alkalinity of the borohydride reagent renders unsatisfactory the determination of formaldehyde liberated after reduction and selective oxidation with periodate.

Trisaccharide II also possessed the capacity to inhibit a number of anti-*A* reagents, and it is noteworthy that in tests for inhibition of sheep cell hæmolysis, it was rather less active (Table 1) than trisaccharide I. Trisaccharide II is believed to be *O*-α-*N*-acetylgalactosaminoyl-(1 → 3)-*O*-β-galactosyl-(1 → 3)-*N*-acetylglucosamine.

The long-known and unique association of group *A* antigens with both *iso*-agglutinins and heterophile (Forssman) antibodies[8] seems to depend on the similar specificities of these antibodies. It seems probable that they correspond to different but overlapping parts of the same carbohydrate structure, the common part being represented by *O*-α-*N*-acetylgalactosaminoyl-(1 → 3)-galactose. This relationship will be discussed in more detail elsewhere. The association of each of the trisaccharides with these two serologically different reagents strengthens the belief that they arise from specific determinant groups in the macromolecule rather than from latent structures which are not directly concerned with specificity.

We wish to thank the Nuffield Trust for financial support. One of us (I. A. F. L. C.) thanks the Lister Institute for a studentship.

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THE FEULGEN-STAINED SOMATIC CELL NUCLEUS AS A HOMOGENEOUSLY ABSORBING OBJECT

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IT is generally accepted that a somatic cell nucleus doubles its genetic material between two consecutive divisions, that the deoxyribonucleic acid (DNA) content of the nucleus is identical with, or at least proportionate to, the nuclear amount of genetic material; further, that the Feulgen reaction is specific for the nuclear DNA, and finally, that the intensity of the Feulgen stain is proportionate to the amount of DNA. So by measuring the intensity, E, of the Feulgen stain of a nucleus and multiplying E with the squared nuclear diameter, D^2, it is imagined that a direct measure of mass is obtained. This is seemingly corroborated by the finding by numerous investigators that the ED^2 values from haploid, diploid, tetraploid and octoploid nuclei show modal distributions with modes at (approximately) 1×, 2×, 4× and 8× ED^2. In this article it will be shown that ED^2 is not a direct measure of mass.

The interpretation of ED^2 as a direct measure of mass would be correct if the nuclei were homogeneously absorbing objects, that is, objects in which the absorbing particles are completely randomly distributed, non-interacting, and retaining their size and shape. For such a system the absorbance, E, is a linear function of the number of absorbing particles, C, the path-length, L, and the molecular absorbancy index ε, which latter can be omitted when dealing with relative amounts of the same compounds. As the concentration is mass per volume (M/V) and as the path-length may be taken as being equal to the diameter, the absorbance of a homogeneously absorbing sphere will be:

$$E = \frac{MD}{1{\cdot}33\pi r^3} = 1{\cdot}92\ MD^{-2} \qquad (1)$$

So by plotting E against D^{-2} a straight line starting in origin and with slope of 1·92 M should be obtained. As a paradigm of the straight line course, Alfert's[1] data on rat liver nuclei have been plotted this way in Fig. 1. From Table 1, where the results of regression analyses of different investigators' published data are given, it is seen that in seven out of nine cases the numerical value of the constant a deviates significantly from zero. This indicates that the relationship between E and D^{-2} most frequently is:

$$E_m = a + bD^{-2} \qquad (2)$$

If the nuclei were homogeneously absorbing objects the occurrence of the constant a would mean that the measured absorbance, E_m, is the sum of the absorbance of the compound in question, E_r, and a constant non-specific absorbance, thus:

$$E_m = E_r + a \qquad (3)$$

XUM

Table 1

	Cell type	No.	$y = a + bx^{-2}$	$P\{|a|\}$	
Alfert* *et al.*	Diploid (liver)	30	$E = -0{\cdot}026 + 15{\cdot}67\ D^{-2}$	0·0025 – 0·001	(3*a*)
	Tetraploid (liver)	30	$E = 0{\cdot}042 + 27{\cdot}36\ D^{-2}$	$\leqslant$ 0·0005	(3*b*)
	Octoploid (liver)	20	$E = 0{\cdot}014 + 54{\cdot}17\ D^{-2}$	0·25 – 0·1	(3*c*)
	Thyroid (control)	28	$E = -0{\cdot}019 + 15{\cdot}14\ D^{-2}$	0·10 – 0·05	(3*d*)
	Thyroid (experimental)	30	$E = 0{\cdot}237 + 10{\cdot}35\ D^{-2}$	$\leqslant$ 0·0005	(3*e*)
Pasteels* and Bullough	Untreated epithelial nuclei	30	$E = 0{\cdot}120 + 11{\cdot}86\ D^{-2}$	$\leqslant$ 0·0005	(3*f*)
	Treated epithelial nuclei	41	$E = 0{\cdot}092 + 15{\cdot}93\ D^{-2}$	$\leqslant$ 0·0005	(3*g*)
Swift	Neuron	10	$E = 0{\cdot}014 + 15{\cdot}21\ D^{-2}$	0·05 – 0·025	(3*h*)
	Liver	10	$E = 0{\cdot}163 + 9{\cdot}35\ D^{-2}$	$\leqslant$ 0·0005	(3*i*)

* Dr. Alfert and Dr. Pasteels have kindly sent me their original data.

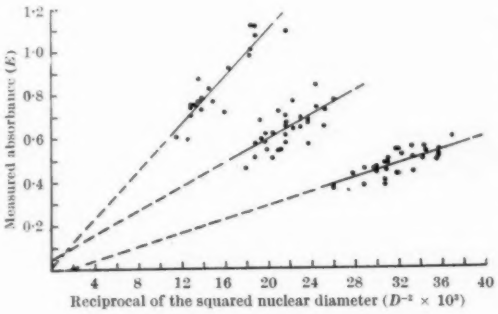

Fig. 1. Alfert's (1955) data for rat liver nuclei

The mass of the compound in question will therefore be:

$$E_r D^2 = (E_m - a)D^2 \qquad (4)$$

and from equation (2):

$$(E_m - a)D^2 = (a + bD^{-2} - a)D^2 = b \qquad (4a)$$

The mass remains constant, as is also obvious in the case of equation (1), where:

$$E_m D^2 = E_r D^2 = 1{\cdot}92\ M \qquad (5)$$

However, some investigators have claimed that their photometric data show synthesis to occur. Here it will be demonstrated that the reason for these claims seems to be that the mass has been calculated as $E_m D^2$ instead of $E_r D^2$.

Thus Swift[7] claims to have demonstrated that synthesis occurs in embryonic mouse liver nuclei. The majority of his data are given as histograms showing the number of nuclei of a given nuclear 'mass'. He also gives, however, the raw data of E and D for 10 measurements on embryonic mouse liver, which presumably are representative for the rest of the measurements on this tissue. From Fig. 2 where E is plotted against D^{-2} it is seen that the data conform to a straight line course with a significant y-intercept (equation 3*i*). If the mass in this case is calculated as $E_m D^2$ and plotted against D the resulting curve, shown in Fig. 3, might be thought to show that the mass increased with increasing diameter, that is, that synthesis does take place. The correctly calculated mass would, however, be $E_r D^2 = b$. The mass remains constant, indicated in Fig. 3 by line B, and no synthesis occurs.

Walker and Yates[8] measured the light absorption in the ultra-violet and visible regions of living and Feulgen-stained nuclei of chick heart tissue cultures at known times after mitosis. The total amount of absorbing matter, which for the sake of convenience here will be called 'mass', was calculated as $E_m D_T^2$, where D_T is the nuclear diameter at time T after mitosis. It should be emphasized that a given nucleus was measured at only one time after mitosis, the same nucleus was not measured at known different times after mitosis, from which it follows that the additional information about the intermitotic age of the nuclei does not make these measurements different in any way from measurements on nuclei of unknown intermitotic age. In both cases the relation between

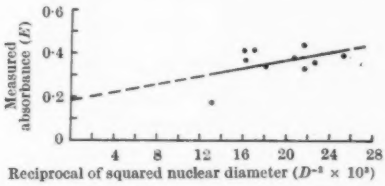

Fig. 2. Swift's (1950) data for embryonic mouse liver nuclei

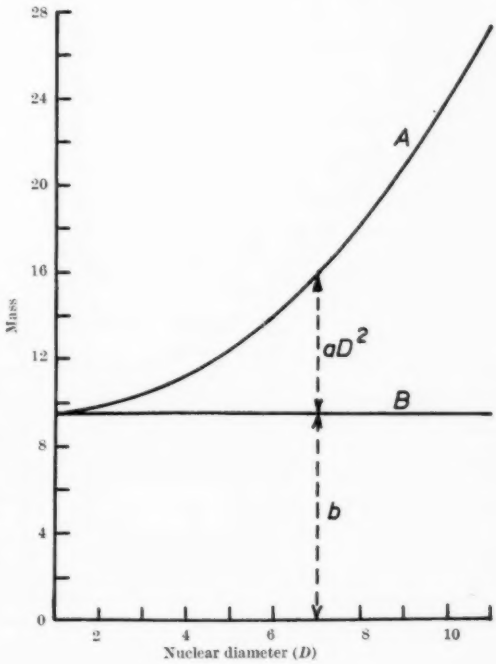

Fig. 3

E and D^{-2} is completely independent of time, inhomogeneity of the population and non-random selection.

Walker and Yates plot mass against time after mitosis. Obviously the course of such curves must be determined by the way in which the nuclear diameter alters with time. If we assume in conformity with the vast majority of data on growth[2] that the increase in geometrical dimensions (= 'growth') of a nucleus is an exponential function of time, or, in other words, that the diameter is increasing in time with a rate which is proportional to the diameter, we have:

$$D_T \sim e^{KT} \qquad (6)$$

Thus plotting $E_m D^2$ (= 'mass') against time would be equivalent to plotting $E_m D^2$ against ln D_T. In Figs. 4 and 5, ln $E_m D^2$ is plotted against time, the experimental points being read off Walker and Yates's graphs of $E_m D^2$ against T for the total ultra-violet absorption and for the Feulgen-stained material, respectively. As is seen from these figures, the regression lines which have the equations:

$$\ln E_m D^2 = 1{\cdot}903 + 0{\cdot}035\ T \text{ for Fig. 4}$$

and

$$\ln E_m D^2 = 1{\cdot}393 + 0{\cdot}077\ T \text{ for Fig. 5}$$

fit the data well.

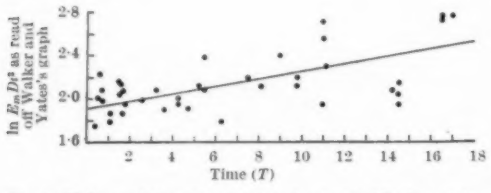

Fig. 4. Walker and Yates's (1952) data for the total Feulgen stain intensity

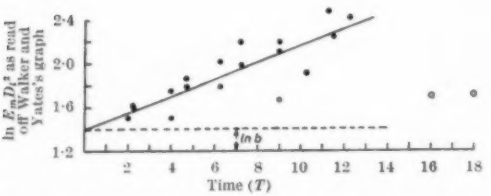

Fig. 5. Walker and Yates's (1952) data for the total ultra-violet absorbance

A plot of ln $E_m D^2$ against $2KT$ ($\simeq$ ln D_T^2) would also result in a straight line, but having half the slope of the lines in Figs. 4 and 5. Consequently, a plot of $E_m D^2$ against D^2 would result in curves which were upwards concave and having y-intercepts of 6·69 and 4·01, respectively. The correctly calculated 'mass' would therefore be:

$$E_m D^2 = (a + bD^{-2} - a)D^2 = b \qquad (7)$$

where $D^2 \sim e^{2KT}$, giving constant masses of 6·69 and 4·01 respectively. It should be pointed out that even if the nuclear growth were not exponential in time, but was another function of time, a linear transformation of such function would lead to similar results.

The claims of having demonstrated, on the basis of photometric data, that synthesis takes place during interphase cannot therefore be said to be proved. If homogeneity is assumed and if the data conform to equations (1) or (2) an increase of M is indeed an impossibility.

This is also evident from the following argument: if the chromosome number ($\sim$ 'mass') doubles during interphase, a tetraploid nucleus at the beginning of the interphase would have the same mass as a diploid nucleus at the end of the interphase. We would therefore expect, if the nuclei were homogeneously absorbing objects, that the absorbancy of nuclei of different ploidy, when plotted against D^{-2}, would all fall on the same straight line. But as seen from Fig. 1, and from Table 1, straight lines with definitely different slopes are obtained, giving different absorbances for nuclei of identical geometrical dimensions and (presumably) mass.

It could then be argued that the doubling of the nuclear genetic material takes place when the nuclei are in a phase which is not represented in the population of measured nuclei, that is, during the mitotic process. The nuclear interphase mass (= Feulgen stain intensity) would remain constant and if the absorbing particles were randomly distributed, E would be linear with D^{-2}, as observed. There are, however, two reasons for not accepting this interpretation. The first is that chemical estimation of DNA (ref. 5) shows the synthesis to occur during the interphase; and the other is that in fixed nuclei the Feulgen-stained material (and nucleoli) is distributed in a peripheral zone[3,4], the peripheral edge of which cannot be distinguished from the nuclear membrane. Therefore, calculation of nuclear mass from photometric data is not possible until such parameters as thickness, possible concentration gradient of the stained particles in this zone, and possible orientation of the absorbing particles in this zone can be estimated.

Further, if the population of measured nuclei is representative of the true population of interphase nuclei, an assumption which is tacitly granted, and if the mitoses in the parent tissue do not occur synchronously, but randomly, and if all the nuclei follow the same basic growth equation, then the probability of including any given nucleus of any given interphase age in the measured population is constant and identical for all the nuclei irrespective of the type of the underlying kinetic equation. If ED^2 were a direct measure of mass we should not expect, therefore, to observe a modal distribution of these values.

We must conclude, therefore, that ED^2 is not a direct measure of mass and the seemingly good agreement between these values and chromosome numbers must therefore be due to some other cause.

I wish to thank Dr. P. R. Peacock for valuable discussion and for reading and criticizing the manuscript.

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REGENERATION OF ELEMENTS OF DONOR ORIGIN IN ORTHOTOPICALLY GRAFTED SKIN FOLLOWING THE HOMOGRAFT RESPONSE

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THE work reported here arose out of attempts to induce immunological tolerance of orthotopic skin grafts in adult inbred mice by the administration of 6-mercaptopurine (6-MP). Groups of recipient mice were treated with 0·25, 0·50, 1·0, 1·5 and 2·0 mgm. of 6-MP daily for 7–10 days before grafting, and for several days thereafter. In order to provide a more generalized antigenic stimulus, several groups were also injected intraperitoneally with isolated spleen cells of the graft-donor strain during treatment with 6-MP. However, the mean survival-time for *C57B1* homografts on *C3H* mice was only prolonged by 3 days by these procedures and this effect was only observed in recipients in which gross lymphoid involution had been produced by toxic doses of 6-MP. The survival of *C3H* skin grafted on to *C57B1* mice was not prolonged. The results of these experiments are in agreement with the findings of Meeker *et al.*[1]. They failed to observe prolongation of homograft survival in mice treated with 6-MP. Similar experiments in which grafts were exchanged between the closely related *AKR*, and *C3H* mice treated with 6-MP, gave the same negative result. In the course of this work, we observed in a number of the mice which had been pre-sensitized with spleen cells that homografts survived indefinitely if the removal of the plaster protective bandages was delayed until 16–17 days after grafting. This indefinite survival occurred in mice that had received 6-MP and in others which had not received the drug. The present article is concerned with this phenomenon of the apparent survival of skin homografts in specifically immunized mice.

In a typical experiment six *C3H* adult mice were each injected intraperitoneally three times during a 10-day interval with 20×10^6 *C57B1* nucleated spleen cells and grafted with *C57B1* skin by Medawar's technique 5 days after the third course of injections. A control group of 6 *C3H* adult male mice which were not treated received *C57B1* grafts at the same time as the injected group. The protective bandages were removed from both control and treated animals 16 days after grafting. The grafts on the treated animals, though non-pigmented and showing no hair growth, were retained without gross change in appearance for an indefinite period. The grafts on the control animals had a similar appearance when the bandages were removed, but rapidly dried out on exposure to the air, and were afterwards completely rejected. The surviving grafts were retained for several months, although macroscopic pigmentation and normal hair growth did not occur. Similar results have been observed when skin homotransplants have been transferred to specifically treated animals in other strain-combinations (*C3H* and *C57B1* grafts on appropriately immunized *AKR* mice). Attempts to extend this phenomenon to inter-species graft survival (rat skin on mouse, mouse skin transplanted to rats) have failed.

Although the response observed in *C3H* mice pretreated by injection of cells differs sharply from the response observed in untreated animals at 16 days this difference was not so apparent when the plaster bandage was retained intact for a further 4–5 days. A proportion of homografts remained intact on control recipients when the protective bandages were removed at 20 days. The possible explanation of this unexpected finding was sought by histological examination of the development of the homograft reaction in pretreated and untreated mice. Histological sections of grafts were prepared at 2-day intervals following the orthotopic transfer of *C57B1* skin to treated and untreated *C3H* recipients. Two distinct kinds of cellular infiltration were observed in sections obtained from both treated and control animals. The more superficial band, predominantly polymorphonuclear, developed immediately below the epithelial layers of the grafted tissues and, concurrently, a deeper band of infiltrating polymorphs and lymphocytes demarcated the graft tissue from the panniculus carnosus of the host. The development of the destructive sub-epithelial infiltrate was an acute event in both treated and control mice. The only obvious difference in response in the two groups of animals was that the sub-epithelial accumulation of polymorphs was most intense in the treated group of mice at 4–6 days, while this response was delayed for 8–10 days in the control animals. The demarcating inflammatory response at the deeper level also developed at 4–6 days in treated animals, and at 8–10 days in the controls, but afterwards followed a more chronic course in both groups. By about the third week after grafting, the demarcating zone between graft and graft bed was characterized by a mononuclear infiltrate containing primitive reticulum cells, lymphocytes and plasma cells and fibroblastic elements. Few polymorphs were then evident in this zone. With the subsidence of the acute polymorph response, the graft epithelium began a process of regeneration which was essentially complete in 10 days; that is, 14–16 days after grafting in the treated group, and correspondingly later in that proportion of the control animals in which regeneration occurred, that is, at 18–20 days after grafting.

Perhaps the most significant feature of these findings is that the sub-epithelial infiltration of polymorphs did not recur on the regeneration of the epithelium in either group. This failure of the host to react against the newly established epithelium has usually been interpreted as indicating that the regeneration is by cellular elements wholly derived from the host. There are difficulties in accepting this view, and we have therefore considered three

Table 1. Transfer of Regenerated Tissues

Strain of skin donor	Recipient strain and No.*	Time of transfer of regenerating graft and strain of recipient	Strain of donor of challenge graft	Histological appearance of challenge graft at 6 days —classification of immunological response
C57B1	10 × *C3H*	*C3H*—16 days	*C57B1*	*Epithelial layers*—some areas of old necrotic epithelium remaining; 1–4 layers of regenerating epithelial cells showing mitotic activity. *Sub-epithelial infiltrate*—single lymphocytes and/or polymorphonuclear debris. *Demarcating infiltrate*—intense lymphocytic and fibroblastic with polymorphs mainly in and around the vessels. *Classification*—second set homograft response.
C57B1 *C57B1*	6 × *C3H* 6 × *C3H*	*C57B1*—17 days *AKR*—16 days	*C3H* *C57B1*	As above. As above.
C57B1	4 × *C3H*	*AKR*—16 days	*C3H*	*Epithelial layers*—large areas of destroyed epithelium remaining; 1–2 layers of regenerating epithelium showing mitotic activity in remainder of grafts. *Sub-epithelial infiltrates*—as above. *Demarcating infiltrate*—as above. *Classification*—second set homograft response.
C57B1	4 × *AKR*	*AKR*—18 days	*C57B1*	*Epithelial layers*—epithelium completely destroyed; no regeneration observed. *Sub-epithelial infiltrate*—intense polymorphonuclear infiltration. *Demarcating infiltrate*—moderate polymorphonuclear, lymphocytic and fibroblastic; some plasma cells. *Classification*—second set homograft response.

* All recipient animals were injected intraperitoneally with 20×10^6 nucleated spleen cells of the skin donor strain three times during the 10-day period before grafting.

other possibilities. The failure of response might also be due to: (*a*) a change in the general immunological status of the host; (*b*) a change in the local immunological status of the host; or (*c*) failure of regenerating donor tissue to attain antigenic competence either by 'adaption' or by deletion of antigenic determinants followed by a process of local selection for survival. A change in the general immunological status of the host is excluded by the observation that a second set graft reaction is invoked in these animals by a second graft with the skin of the donor (*C57B1*) strain. The possibility that the graft has not regained antigenic competence of donor strain specificity can be experimentally tested. Transfer of the regenerated tissue to normal mice of the recipient (*C3H*) strain invokes a first set homograft reaction as judged both macroscopically and microscopically. It has not been possible to include as a control an equivalent area from a *C3H* mouse in which regenerating epithelium of known host origin has replaced the normal skin, but the validity of our observation is supported by the fact that mice having rejected the graft of regenerated tissue were afterwards able to reject a further *C57* graft with a typical second set, accelerated reaction (Table 1).

This is *prima facie* evidence that a regenerating element in the transferred graft has donor (*C57B1*) specificity, and can sensitize the new recipient to give a second set response. It is quite unlikely that either sub-cellular antigenic material or a small proportion of surviving, but inert, epithelial cells of *C57B1* provenance, protected in some way among regenerating host cells, could be responsible for this sensitization. The possibility that presensitization of the recipient mice might be 'adoptively'[2] acquired by the coincidental transfer of isologous, immunologically competent cells present in the regenerated tissue could be tested by transfer in a homologous system where the transferred lymphoid cells would not be expected to survive. Thus *C57B1* grafts surviving on *C3H* mice have been transferred at 16 days to normal *AKR* mice (Table 1). A subsequent *C57B1* graft on these animals stimulated a characteristic second-set graft response, while the appropriate control-*C3H* skin grafted on to normal *AKR* failed to induce a second-set graft reaction when these animals were grafted with *C57B1* tissue later.

Confirmatory evidence that homograft survival in treated mice is related to regeneration of donor elements was obtained by X-irradiation of the donor tissue at a level (1,000 r.) which might be expected to suppress mitotic activity. The irradiation was done as soon as possible after removal of the skin from the donor, the graft being applied to the recipient immediately after irradiation. Despite delay in removal of the protective bandages for 16 days, such grafts were rejected, and this observation is in accord with the hypothesis that regeneration of some elements of donor origin are essential for the graft survival. If regeneration of the graft were wholly due to cellular elements derived from the host then it would be unlikely that moderate X-irradiation of the donor tissue would inhibit regeneration.

There is no doubt that host cells do invade the primary graft. Histologically there is evidence for the entry of connective-tissue elements, and when a surviving graft was transferred to a normal recipient of the donor *C57B1* strain it was destroyed by a typical homograft response. The results we have outlined may be summarized as providing evidence that, in the *C57B1* or *C3H* system, donor cells persist in the homograft for much longer than is now believed, and that if the area is adequately protected, they may regenerate and persist indefinitely despite the recurrence of a homograft reaction. It is much easier to demonstrate this survival if the animals have been pretreated by an injection of the donor type spleen cells, and there are obvious resemblances to the 'enhancement' phenomenon[3,4]. If this is the case, a similar type of homograft survival might be promoted by passive administration of serum from mice which have been hyperimmunized with donor type cells. Accordingly, a group of 16 adult male

C3H mice were grafted with *C57B1* skin. On the sixth day after grafting, concurrent with the onset of the cellular reaction in the first-set graft response, 10 mice were each injected intraperitoneally with 0·2 ml. of serum obtained from *C3H* mice which were hyperimmunized by the intraperitoneal injection of *C57B1* spleen cells at weekly intervals over a period of 6 weeks. Thereafter the grafted mice received intraperitoneal injections of 0·2 ml. of the hyperimmune serum on alternate days until the twentieth day after grafting. The remaining 6 control animals were injected with normal *C3H* serum using the same procedure of route, and dosage, with which the test group was treated with hyperimmune serum. The plaster bandages were removed from both groups of mice at 16 days after grafting, and survival of the grafts in the group injected with hyperimmune serum was both macroscopically and microscopically similar to the homograft survival observed in the actively immunized animals described here. The grafts were destroyed in the control group injected with normal *C3H* serum. This is only a preliminary result but it points strongly towards an enhancement effect. Following Billingham, Brent and Medawar[4], we regard this as due to antibody providing an afferent block in the flow of antigenic material (or information) from the implanting donor cells to the immunologically competent lymphoid centres of the host.

The conclusion to be drawn from our experiments may not necessarily be applicable to any other system than the one we have used, but there is equally nothing to show that they are not of general validity. With this reservation we can formulate them as follows:

(1) A proportion of cells of donor origin survive the homograft response and, provided adequate physical protection is given to the graft, many may survive indefinitely. The cells concerned are not, however, sufficient to conserve the donor material as a functioning tissue; they constitute at most a local chimera containing also a proportion of host cells.

(2) The difference between first and second set reactions is essentially in their timing; second set reactions are initiated and reach their acme 4–6 days earlier than primary ones.

(3) Accepting the orthodox interpretation that homograft rejection is mediated by the soquence: movement of antigenic material from the graft to lymphoid tissue; induction and proliferation of appropriate immunologically competent cells; passage of such cells to the graft site; destructive interaction between immunologically competent cells of the host and the donor cells in the graft, we should add a further component to the sequence, namely, production of circulating antibody which, by intervening between foreign antigenic determinant and receptors on immunologically competent cells at either afferent or effector stages, can diminish or annul the immunological rejection of the homograft.

(4) The intensity and completeness of the homograft response is therefore highly dependent on conditions in the system which modify the degree of physical protection of the graft and on the relative timing and intensity of the cellular and humoral aspects of the immune response.

We are indebted to Sir Macfarlane Burnet for his criticism during the course of this work.

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A NEMIN ASSAY PROCEDURE

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PRAMER and Stoll[1] provided unequivocal evidence that trap formation by the predacious fungus *Arthrobotrys conoides* is induced by a metabolic product of the nematode *Neoaplectana glaseri* and proposed 'nemin' as the name for the substance or group of substances that cause morphogenesis in nematode-trapping fungi. In order to study the chemical nature of nemin and to proceed with isolation and identification of the compound, it was necessary to have a nemin assay procedure. Therefore, the influence of various factors on growth and trap formation by *A. conoides* was determined, and the results were used as a basis for the nemin assay agar and the method described here.

The fungus used as assay organism was isolated from decomposing leaves and identified as *A. conoides*. It is representative of a group of nematode-trapping fungi in which short lateral branches of the mycelium anastomose with similar branches or with the parent hyphæ to form eventually a three-dimensional network of loops. The hyphæ that comprise the loops are covered with a sticky secretion, and nematodes contacting the network are captured by entanglement in and adhesion to the mycelium[2]. *A. conoides* developed readily on a variety of laboratory media, but stock cultures were maintained by periodic transfer on maizemeal-extract agar. Pure cultures of the fungus produced some traps spontaneously, but they were few in number, and no difficulty was encountered in differentiating between control plates on which traps were produced without provocation and treated plates on which trap formation was induced by nemin.

The assay agar was prepared by dissolving 17 gm. of dehydrated maizemeal-extract agar (Difco Laboratories, Inc.) in 1 l. of *M*/45 Sorenson's phosphate buffer at *p*H 5·8. The liquid was brought to the boil and when the agar dissolved 10 p.p.m. of novobiocin and 20 p.p.m. of bromcresol purple were added. The antibiotic prevented bacterial contamination of assay plates. The buffer maintained the reaction of the assay agar at *p*H 5·5–5·8 and thereby kept spontaneous trap formation to a minimum. Bromcresol purple provided visual evidence of any change in the

Table 1. Nemin Activity of Various Blood Sera and Nematode Extracts

Preparation assayed	Highest dilution causing trap formation	Dry weight of preparation (mgm./ml.)	Nemin (units/mgm.)
(1) Sterile pooled normal serum (Colorado Serum Co., Denver, Colorado)			
Cow	1/1,000	86	11·6
Horse	1/2,000	93	21·5
Pig	1/2,000	87	23·0
Sheep	1/1,000	82	12·2
(2) Bovine serum (Hyland Laboratories, Yonkers, New York)			
Liquid	1/500	58	8·6
Dried*	1/1,000	85	11·8
Ultrafiltrate	1/100	15	6·7
Dialysed	1/100	72	1·4
(3) Water extracts of nematodes			
Panagrellus redivivus	1/1,000	5	200·0
Pig ascarids	1/1,000	27	37·0
(4) Culture broth of *Neoaplectana glaseri*†	1/100–1/1,000	37	2·7–27·0

* Reconstituted in sterile distilled water saturated with CO_2.
† Obtained from Dr. N. R. Stoll, of the Rockefeller Institute, New York.

reaction of the medium caused by material incorporated into the agar for assay or by growth of the fungus. The antibiotic, buffer, and *p*H indicator at the concentrations employed had no influence on growth or trap formation by *A. conoides*. The complete assay agar was sterilized by autoclaving.

Solutions for assay were sterilized by filtration through membranes or sintered glass and diluted in sterile water blanks. One-ml. quantities thereof were added to separate Petri dishes and mixed thoroughly with 9 ml. of the assay agar poured at approximately 45° C. When the agar had solidified, the centre of the surface of each assay plate was inoculated with a plug having a diameter of 4 mm. and cut with a cork borer from those portions of a maizemeal-extract agar plate on which the growth of *A. conoides* was 1–2 days old. All assays were made in triplicate. Results were obtained by microscopic examination (× 100) of the mycelium that developed during 48 hr. storage at 28° C. Observation was neither tedious nor prolonged since the amount of growth made by the assay organism was relatively scant and traps were easily distinguished.

The total solids content of preparations assayed was determined by drying an appropriate quantity at 105° C. for approximately 18 hr. Nemin activity was measured in terms of dilution units/mgm. The nemin unit was the reciprocal of the highest dilution of the preparation that caused the fungus to form traps. If a preparation had a total solids content of 10 mgm. and was active at a dilution of 1 : 1,000 it contained 1,000 dilution units/10 mgm. or 100 units/mgm.

The assay procedure as described was used to determine the nemin activity of various animal sera, aqueous extracts of two nematodes, and culture broths of the nematode *N. glaseri*. It is apparent from the results summarized in Table 1 that water extracts of the free-living nematode *Panagrellus redivivus* had the greatest nemin activity. Sera derived from the cow, horse, pig, and sheep had comparable activity, and dried bovine serum did not differ greatly in nemin content from normal serum. The nemin activity of an ultrafiltrate of bovine serum was similar to whole serum, indicating that the size of the nemin molecule is not too great to pass through 'Cellophane'. This was verified by dialysing bovine serum against distilled water for 2 days. The whole serum had a nemin activity of 8·6 units/mgm., but after dialysis it decreased to 1·4 units/mgm. The loss of nemin from blood serum during dialysis supports the statement of Pramer and Stoll[1] that nemin is not a protein or polysaccharide of high molecular weight.

Since chemical fractionation and isolation work frequently yield small quantities of material, a microassay method was developed and tested. The same assay agar was employed and 10-ml. quantities were added to sterile Petri dishes. When the agar had solidified the plates were inoculated as described above and incubated at 28° C. for 5 days. Holes (5 mm. diameter) were punched from the centre of absorbent paper disks 13 mm. in diameter and 5 disks were placed equally distant from each other around the circumference of the agar surface in each plate. The disks were located such that the mycelium exposed through the centre holes was 1–2 days old. The preparations assayed were diluted serially in water and 0·1 ml. aliquots of the solutions added directly to the fungus mycelium visible through the holes in the centre of the absorbent disks. All tests were made in triplicate and the plates returned to the incubator for 36 hr. The treated areas were then examined microscopically for traps and nemin activity measured as dilution units/mgm. The micromethod was found suitable for locating nemin on paper strip chromatograms. For this purpose the strips were cut into 2-cm. lengths and these were substituted for the absorbent disks.

The micromethod requires 0·1 rather than 1·0 ml. of solution for assay. Moreover, 5 determinations instead of 1 are made per plate and results are read after 36 rather than 48 hr. Repeated tests have shown that both assay procedures yield comparable results. The nature, quantity of material, and time available for assay dictate which of the two methods is the more suitable. A dilution assay such as that developed for the estimation of nemin is limited in its sensitivity since measurements can be made only between concentrations of the dilution series tested. Precision can be increased by using more narrow increments in a dilution series, but this necessitates an increased expenditure of time and effort. In the nemin assay the preparations to be tested were sterilized. Although this is not a requirement of the micromethod, results are more reproducible and clearly defined when aseptic technique is employed. In spite of these disadvantages which are inherent in all serial dilution assays the procedure remains a useful and important means of measuring the activity of biologically active compounds[3]. Repeated tests have shown that the results obtained with the nemin assay are reliable and reproducible and the method as developed is suitable for the control of fractionation studies for the chemical isolation and identification of nemin. For this purpose pig ascarids are being used. These worms are numerous, individually large, and obtainable in quantity from slaughter houses. The results listed in Table 1 show that water extracts of pig ascarids have greater nemin activity than animal sera and culture broths of *N. glaseri*.

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LETTERS TO THE EDITORS

RADIOPHYSICS

Group and Phase Velocities of a Travelling Disturbance in the *F* Region of the Ionosphere

A PUZZLING feature of the reported association[1] between travelling disturbances in the *F* region and the occurrence of sporadic *E* is that travelling disturbances move with a velocity of about 10 km./min. (ref. 2) whereas sporadic *E* patches have only about half this velocity[3]. However, the travelling disturbance velocity was measured using widely spaced stations (~ 1,000 km.) whereas that of the sporadic *E* patches was found using relatively close stations (~30 km.). Therefore whereas it is the group velocity of the travelling disturbances which has been measured, the sporadic *E* patches may be associated with a particular phase of the isoionic contours in the travelling disturbance and may thus appear to move with the phase velocity of the travelling disturbances.

To test this hypothesis, three methods have been used for measuring directly the phase velocity of a travelling disturbance, making use of existing records. The ionograms taken at one station have been reduced to give isoionic curves plotted against time. These curves are then used in the following ways:

(1) The radius of curvature of the isoionic contours in space may be estimated from the observed splitting of the echo. For example, when the second echo is split, but the first is not, the radius of curvature is between once and twice the height of the contour. When the radius of curvature at any time is known, the phase velocity may be estimated.

(2) The second echo virtual path is often less than twice the virtual path of the first echo, because of the different paths followed. If the isoionic contours are assumed to be concentric circles over short distances, it is possible to calculate the difference in terms of known quantities and the phase velocity.

(3) The wave-fronts of travelling disturbances are believed to be approximately along the Earth's magnetic field[3,4]. If this is assumed to be correct, a further estimate of phase velocity may be made using the isoionic curves.

One set of two-minute records taken at Brisbane has been found suitable for analysis. The group velocity of the disturbance was found from ionograms at this and other stations to be 10 km./min. All three methods gave a phase velocity between 4 and 6 km./min., confirming the hypothesis.

The difference in group and phase velocities may also explain the short apparent life-time (~30 min.) of phase-path irregularities[5] and the long life-time of disturbances on ionograms at spaced stations (~5 hr.). The form of the phase path irregularities changes rapidly due to the large dispersion.

'Satellite' traces on ionograms are due to sloping isoionic contours which are observed to move with a velocity of ~4 km./min. (ref. 6). Because of the method used for measuring it, this velocity is evidently the phase velocity. Thus the one obstacle to the identification of the cause of satellite traces as travelling disturbances, that is, their different velocities, is removed.

The travelling disturbances discussed here are those which cause gross distortions of ionogram records. Small disturbances on $h' : t$ records have been shown to move with speeds of nearly the same magnitude[7], but differing in direction by about 20°. This may be due to the influence of the phase velocity, which may have an east–west component not appearing in the group velocity which is nearly due north.

The reported dispersion does not agree with that given by Hines[8] for internal gravity waves which he regards as the cause of travelling disturbances.

Further details of the experimental method will be presented elsewhere.

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OCEANOGRAPHY

Evidence of an Eastward Equatorial Undercurrent in the Atlantic from Measurements of Current Shear

RECENTLY there has been speculation as to the existence of an equatorial countercurrent in the Atlantic Ocean similar to the Cromwell Current[1,2] in the Pacific. G. Neumann[3] has cited evidence for such a current from early observations (1911) of anomalous drift currents and from a re-analysis of dynamic heights from oceanographical data along the equator. In the first two weeks of March, 1961, during an extensive bathymetry survey of the mid-Atlantic Ridge along the equator, I was able to make a number of measurements of horizontal current shear in the upper 100 m. surface water. The shears obtained at different points over an area extending from 19° W. to 10° W. longitude and from the equator to 3° S. latitude do indicate the presence of an eastward flowing equatorial undercurrent. The measurements were made from the R.V. *Chain* during the first part of a three-month cruise to study oceanographical problems associated with the Romanche Trench and the equatorial mid-Atlantic Ridge.

The current shear was measured by a method suggested to me by W. S. Richardson of this Institution. Two pitotmeters were mounted at the top and bottom, respectively, of the 600-ft. towed chain which carried thermistor elements for the continuous temperature profiling system[4]. When the ship was under

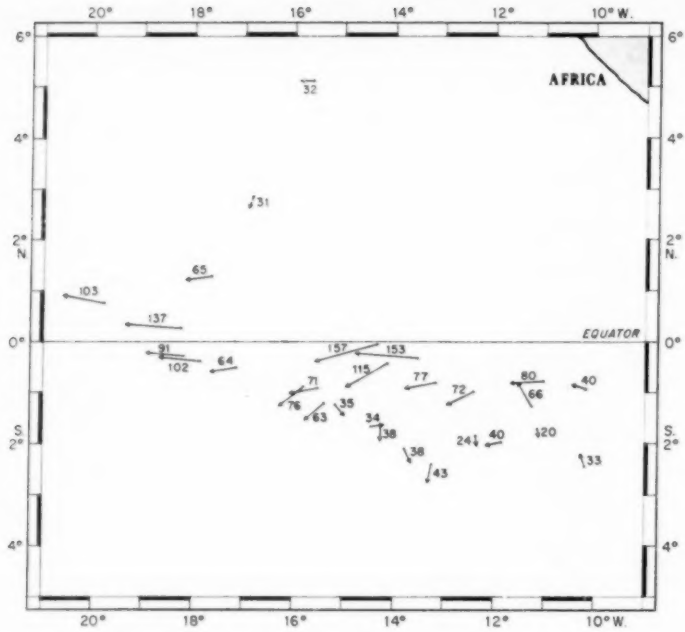

Fig. 1. Horizontal current shear [$\vec{V}$ (15 m.) − $\vec{V}$ (105 m.)] measured near the equator during March 4–21, 1961 (shear magnitude in cm./sec. indicated beside each vector)

way at a normal speed of 11 knots the shallow pitotmeter was at a depth of 15 m. and the deep pitotmeter at a depth of 105 m. The water speed past each pitotmeter was determined electrically from a differential pressure transducer driven by the pitot. By manoeuvring the ship at constant speed (11 knots) around a box course I could measure the vector difference between the current velocity at the pitotmeter and the drift velocity (wind drift and surface current) of the ship at the surface. By subtracting the result of one pitotmeter from that of the other I could determine the vector difference or shear between the current velocities at the two pitotmeters.

The preliminary results of shear measurements are shown on the chart in the accompanying Fig. 1. Each vector represents a measurement and gives the magnitude and direction of the difference: $\left[\vec{V}\text{ (15 m.)} - \vec{V}\text{ (105 m.)}\right]$, where $\vec{V}$ (15 m.) is the current velocity at a depth of 15 m. and $\vec{V}$ (105 m.) is the current velocity at 105 m. The magnitude of the shear in cm./sec. is indicated beside each vector. Clearly apparent in Fig. 1 is the unusually strong westerly shear of the 15-m. water over the 105-m. water along the equator. This shear was as high as 150 cm./sec. in two places. The shears shown give no information, of course, about absolute current velocities. However, determinations of the set of the ship while under way and while drifting indicate a current velocity at the surface of 40–60 cm./sec. to the westward at the equator. Because the current at 15 m. was about the same as that at the surface it is clear from the shears shown that the undercurrent at the 105-m.-level is toward the east along the equator and at some points as high as 100 cm./sec. The presence of this undercurrent was indicated very clearly during the lowering of a plankton net prior to a tow. When the net reached a depth of about 50 m. the wire angle changed drastically and stood out from the ship towards the east.

The shears shown in Fig. 1 indicate that the southern boundary of the undercurrent in these longitudes lies somewhere between 1° and 2° south latitude. The few measurements north of the equator show very little concerning the northern boundary of the undercurrent, but suggest that the current is more or less symmetrical about the equator. The thermal structure of the water, which was recorded continuously throughout the survey to a depth of about 110 m., underwent an interesting transition whenever the ship crossed the boundary of the current. South of the current most of the total drop in temperature from 28° C. at the surface to 15° C. at 110 m. was concentrated within a very strong negative gradient at a depth of about 50 m. This gradient is the well-known equatorial discontinuity layer. Along the equator, however, the drop in temperature was distributed evenly from the surface to 110 m. with an approximately constant negative gradient.

This work was carried out under contract with the Office of Naval Research (*Nonr*-1367).

Measurements of the undercurrent are continuing aboard the R.V. *Chain* in this area at the time of writing. Detailed measurements of the undercurrents velocity profile are being made using current meters and current drogues by C. Parker.

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GEOLOGY

Ultrabasic Liquids

THERE is a considerable body of geological evidence suggesting that, in a submarine environment, extrusion of silicate liquids with a composition comparable to peridotite occurs. Later, this material is hydrated to form serpentine. In part, this evidence comes from repeated association of serpentines with marine sediments such as greywackes and cherts, and repeated association with volcanics including pillow lavas. Further, the ultramafics of this environment show features to be expected from rocks which have flowed. An excellent description of such a situation is provided by Bailey and McCallien[1]. Hess[2] has summarized some of the data from field evidence suggesting that many ultrabasic rocks were formed

by intrusion of liquids. But laboratory investigation has consistently heaped difficulties on an extrusive explanation of such rocks. Known surface lava temperatures do not exceed 1,200° C. while the melting temperatures of the anhydrous equivalents of serpentine are of the order of 1,700° C. Clearly there are difficulties in moving such materials to the surface of the Earth, or even in forming liquids at reasonable depths in the crust. The following statement from Hess[2] bears repeating: "For most of this century a magnificent argument has gone on between field geologists who have worked on the peridotites of alpine mountains and laboratory investigators of their chemistry (particularly Bowen). The former stoutly maintains that the evidence indicates that they were intruded in a fluid state, as magmas; and the latter equally forcefully has proved to his satisfaction that such magmas are not possible. . . . At the other extreme we find Bailey and McCallien suggesting that the serpentines of Turkey are submarine lava flows."

Recent work has shown that submarine volcanic activity is widespread but the consequences of this have received insufficient attention. Under moderate pressures of water, the melting points of rocks and minerals are normally depressed several hundred degrees. When a lava is extruded at the surface, it cannot contain volatiles at pressures much in excess of atmospheric pressure. When the same material is extruded in a submarine environment, volatiles can be retained at pressures corresponding to the water pressure of the given depth. It follows that submarine lavas will occur at much lower temperatures than their surface equivalents. If silicate liquids corresponding to compositions with high melting points are to be expected as extrusives, the most likely environment will be that of the deep oceans.

With these considerations in mind, a series of preliminary experiments on the melting points of serpentine compositions have been conducted under water pressures of 500–1,000 atm., corresponding to the deeper parts of the oceans. The material chosen was a typical serpentine from a typical 'flow' environment in New Zealand. Hydrothermal treatment indicated that above 500° C., the serpentine was replaced by talc and forsterite. At higher temperatures, forsterite, enstatite and minor chromite were dominant. Between 900–1,200° C., pyroxene and olivine were fine-grained, but near 1,300° C. the first suggestions of melting were indicated. By 1,400° C. it was clear that most of the material was liquid during the experiment. Residual olivine was coarse-grained; glass was present together with masses of feathery growths of olivine or pyroxene. This latter growth habit is typical of that in greatly supersaturated environments and probably took place during quenching, a matter of a few seconds. Samples also suggested that considerable flow of material had occurred.

It thus appears that serpentine lava could flow at temperatures of about 100° C. above observed surface lava temperatures, in a submarine environment. In our opinion, such temperatures cannot be considered beyond the range of geological possibility. At greater depths, intrusion of partially melted ultramafic materials should be possible at even lower temperatures. Our quenching results also indicate that well-quenched margins should be rare.

It has been noted frequently that ultrabasic rocks seldom show thermal effects at their contacts, which might be anticipated from their melting temperatures (for a summary, see Turner and Verhoogen[3]). In the case of submarine flows, no contact metamorphism would be anticipated. But even in deeper intrusions it must be noted that the demand for water imposed by the serpentine reaction in chilled marginal phases may cause flow of water into the cooling magma and an abnormally low heating effect at contacts. This situation is in direct opposition to that with granitic rocks where internal water requirements are small, and transfer of water out from the magma may heat contact rocks.

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Preliminary Age-Determinations in the Lizard Area

A petrological re-examination of the Lizard area by one of us (D. H. G.) has led to an attempt to date the igneous and metamorphic assemblages using the potassium–argon method.

Four muscovite samples from the Old Lizard Head series and two samples of biotite from the Kennack gneiss have been measured, using the total volume technique. Determinations of potassium oxide were made using a flame photometer. Results are shown in Table 1. Overall errors are estimated at ± 4 per cent. On geological evidence, the relevant sequence of events in the Lizard is as follows:

Oldest: (1) Regional metamorphism of (*a*) Old Lizard Head series, (*b*) Landewednack hornblende schists, accompanied by the intrusion of the Lizard peridotite. (2) Localized intrusion of gabbro. (3) Intrusion of a basic dyke swarm and related sills, intruded in a stress field approximately at right angles to that operating during the regional metamorphism. (4) Minor intrusions of Kennack granite and injection gneiss. (5) Thrusting of the Lizard block over the Palæozoic sequence to the north.

The age of event (5) is probably Upper Devonian or Lower Carboniferous, but events (1) have been assigned to ages from Archaean[1] to Upper Devonian or Lower Carboniferous[2].

Ages given in Table 1 are not consistent with the established sequence of geological events. Retrograde effects in the biotite–hornblende injection gneiss of the Kennack gneiss are absent or restricted to slight sericitization of feldspar. The specimens of Old Lizard Head series are muscovite–chlorite schists in which the chlorite probably has replaced biotite, and in which cordierite has altered to secondary products. Plagioclase is fresh or shows slight sericitization. Muscovite itself appears quite fresh.

The interpretation placed on these results is that the date given for the Kennack gneiss is a true one, indicating a Caledonian[3] age for the acid intrusion. That obtained for the Old Lizard Head series probably reflects loss of argon caused by slight retro-

Table 1. Results of Potassium-Argon Dating

Material	K_2O (per cent) Average of 5 determinations	Volume of argon (mm.³) / Weight of sample (gm.)	Atmospheric contamination present	δV_R	δt	Age in millions of years
96388 (coarse). Old Lizard Head Series. Pen Olver	9·03	0·1206	2·7	0·4	3·25	357
96388 (fine)	9·01	0·117	1·6	0·2	3·21	352
96391 (fine). Old Lizard Head Series, nr. Life-boat station, Polperro	9·11	0·130	8·4	0·4	3·27	359
96391 (coarse)	9·17	0·121	2·0	0·2	3·20	355 Mean = 356
96392 Kennack Gneiss, Kennack Sands	6·97	0·1188	14·1	0·5	3·58	397
96393 Kennack Gneiss, Polgwidden	5·98	0·0874	3·8	0·6	3·47	384 Mean = 391

$\lambda_\varepsilon = 0{\cdot}584 \times 10^{-10}$ yr.$^{-1}$. $\lambda_\beta = 4{\cdot}72 \times 10^{-10}$ yr.$^{-1}$.
δV_R = Percentage standard deviation in volume of radiogenic argon due to uncertainty in isotope ratios of specimen.
δt = Error in age (in millions of years) due to a 1 per cent error in proportion of K_2O or volume of radiogenic argon.

grade metamorphism during the late (? Hercynian) thrusting.

In order to check the figures yielded by the Old Lizard Head series, determinations are to be made on hornblendes from the Landewednack hornblende schist and from hornblende granulites in the metamorphic aureole of the peridotite.

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CRYSTALLOGRAPHY

Epitaxial Growth of Crystals on One-Degree Orientated Substrates

During the oxidation of one-degree orientated metals, an unexpected variation in the dispositions of oxide crystallites was observed which appears to be similar to epitaxial growth[1] of crystals on single crystal substrate. In this process, the growing crystals (overgrowths) aligned themselves in a certain manner depending on the arrangement of atoms or ions of the substrate and more correctly on the potential energy configuration at or near the surface layers. Consequently a definite orientation relationship is established between their crystal axes, and the overgrowths are said to develop a two-degree orientation such that two axes of theirs are parallel to two of the substrate. This is more often expressed by a plane and axis of each. Single crystal substrates thus greatly influence the orientation of the overgrowths at least in the initial stage of their growth[2]. On different faces of the substrate, the deposit orientation would normally be different, provided the growth condition is not too drastic or temperature not too high. The same orientation may, however, be induced on different faces of the substrate, but is again determined by the substrate structure. Numerous illustrations of such epitaxial growth of crystals are cited in the literature (see Pashley[3]).

On polycrystalline and amorphous substrates, this type of orientated overgrowths is not observed, simply because there is no regular order in the disposition of crystallites of the substrates in the former and no regular periodicity in the atomic arrangement in the latter. In one-degree orientated substrates, however, there is some partial order in distribution of crystallites in the sense that they have a common and parallel axial direction (or plane), known as orientation axis (or plane); but otherwise they are all randomly disposed. No result is yet published which suggests the influence of such substrate on the growing crystals. In view of my observation mentioned earlier, orientated metal substrates were oxidized slowly in air and the nature of the oxide crystals studied by electron diffraction.

Nickel electro-deposits of {100}, {110}, {210} and {10$\bar{1}$0} + {211} orientations (one-degree) and cobalt of {110} were prepared by electro-deposition[4]. These were then heated in air in an electric furnace to a temperature of about 320° C. for about an hour and the surface layers examined by electron diffraction both before and after oxidation in the usual way[2,4].

Table 1

Substrate orientation (1-d)*	Oxide orientation (1-d)*
Ni {100}	NiO {100}
Ni {110}	NiO {110}
Ni {210}	NiO {210}
Ni (10$\bar{1}$0) + {211}	NiO {111}
Ni {Poly}	NiO
Co {110}	Co_3O_4
Co {110}†	CoO {110}

* 1-d: one-degree orientation.
† At about 10^{-2} mm. mercury pressure of air.

Nickel metal was oxidized to nickel monoxide (NiO) only, whereas cobalt to cobalto-cobaltic oxide (Co_3O_4) in air, but to cobaltous oxide (CoO) only at low pressure ($\simeq 10^{-2}$ mm. mercury) of air. The surface layers on nickel substrates consisted not only of orientated polycrystalline monoxide but also of unreacted metal. Nickel monoxide (NiO) crystallites developed different orientations depending on the nature of initial substrate orientation (Table 1). But under the same conditions, polycrystalline nickel was oxidized to unorientated nickel monoxide. On further heating, however, entire surface layers, whether of orientated or unorientated nickel, consisted of polycrystalline nickel monoxide only. Further oxidation would lead to nickel monoxide, having preferred orientation determined entirely by the oxidation conditions similar to cuprous and cupric oxides on copper[5]. For better orientation the oxidation should be carried out at mild conditions. On cobalt {110} surface under milder conditions of oxidation at low pressure, again {110} orientated cobaltous oxide was observed.

These results clearly show that the overgrowth orientations can also be influenced considerably by substrates orientated one degree, at least in the initial stage of growths, and the process closely resembles the epitaxial growth on single crystals.

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GEOCHEMISTRY

Fossil Chlorins in a Triassic Sediment

Spectroscopic evidence for the presence of green chlorophyll-derivatives in ancient sediments and crude oils has been reported by Treibs[1] and by Blumer[2]. We have now succeeded in isolating and characterizing the green pigments responsible for the absorption band at about 640 mμ in the extracts of the porphyrin-rich Triassic oil-shale from Serpiano (Switzerland) (Fig. 1*A*). The pigments are isolated by first removing from the extracts the asphaltenes by iso-octane precipitation and extracting the ether solution of the pigment-concentrate with 6 *N* hydrochloric acid. Subsequent partitioning between ether and hydrochloric acid of increasing concentration removes the stronger bases deoxophyllerythroetioporphyrin (DPEP) and meso-etioporphyrin (MEP). The final purification of the green pigment is then accomplished by repetitive chromatography over partially deactivated alumina (Brockman III). The spectrum of the pure pigment (Fig. 1*B*) is characteristic of the chlorin (dihydroporphin) configuration, and the partial resolution of the band at 490 mμ in addition to the presence of a small band at 612 mμ is indicative of a meso-chlorin, a chlorophyll-derivative in which the vinyl-group in the 2-position has been hydrogenated to an ethyl-group[3]. The high chromatographic mobility of this pigment on alumina and on paper (R_F = 0·75 on Whatman No. 3 paper, carbon tetrachloride–iso-octane developer, 7 : 3, see ref. 4) eliminates the possibility of the presence of acid-, ester- and other polar groups like —COH, —CO, —OH.

The pigment, which is thus characterized as a meso-etiochlorin, can be converted to the corresponding porphin by dehydrogenation with excess chloranil in boiling xylene. The reaction product (Fig. 1*C*) exhibits a typical porphyrin spectrum, intermediate between those of DPEP and MEP and identical with that of the DPEP/MEP-mixture present as uncomplexed pigments in the same sediment. This dehydrogenation product can be resolved by chromatography over alumina into fractions with the etio-spectrum of MEP and phyllo-spectrum of DPEP. Paper chromatography of these fractions confirms the spectroscopic evidence, the R_F-values agreeing within ±0·01 unit with those for pure DPEP and MEP.

For additional structural confirmation, the unknown dihydroderivative of DPEP was synthesized by hydrogenating with Raney-nickel a DPEP concentrate isolated from the oil shale. The spectrum of the hydrogenation product is identical to that of the fossil chlorin. A small difference in the R_F-values

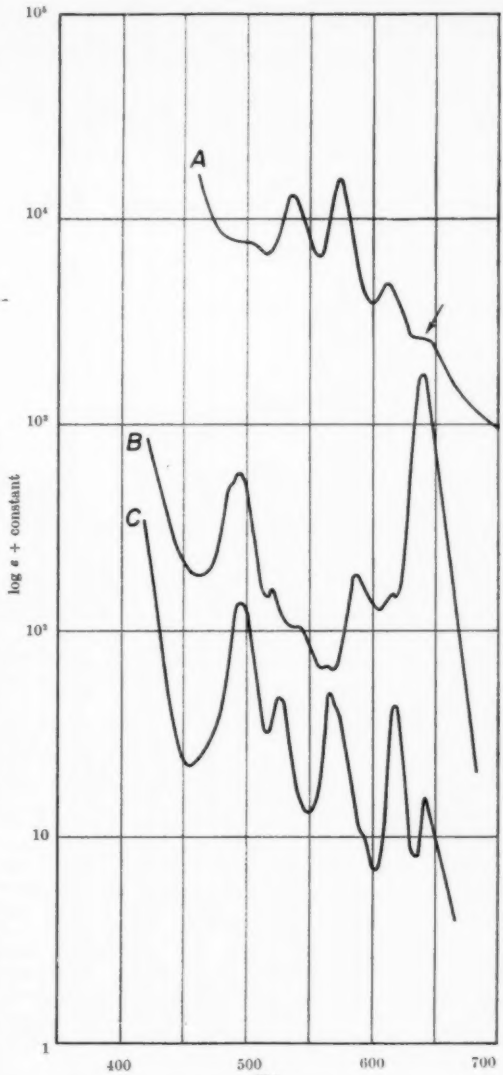

Fig. 1. Spectra. *A*, Benzene–methanol extract of Serpiano oil-shale; *B*, natural dihydro-DPEP containing some dihydro-MEP; *C*, porphyrin mixture obtained by dehydrogenation; the small peak at 641 mμ is a trace of residual chlorin

(0·68 for the synthetic compound, compared with 0·75 for the natural chlorin) is explained by the presence of a larger fraction of the more rapidly moving dihydro-MEP in the natural pigment.

Thus, the fossil chlorin is characterized as a mixture of dihydro-DPEP (deoxo-meso-etio-pyro-pheophorbide *a*) with some dihydro-MEP (etiochlorin III). The etiochlorin must have been formed by intensive reduction from hæmin, protoporphyrin, meso-porphyrin and meso-etioporphyrin being intermediates. The chlorin structure of dihydro-DPEP is also more likely to be of secondary than of primary origin. The vinyl-group of the chlorophyll-pigments disappears early in geological history, probably by isomerization of the vinyl-chlorins to meso-porphyrins.

Any meso-chlorins that might be formed at this stage would be re-oxidized to meso-porphyrins because of their extremely low redox-potential. Only much later, after complete reduction of all carbonyl-groups, can the porphyrin-system be partially reduced with formation of the fossil chlorins.

A more detailed report of this work is being prepared for publication elsewhere. This investigation has been supported by a research contract with the United States Office of Naval Research (Contract *NONR*-2196 (00)) and the grant of a summer fellowship to one of us (G. O.).

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PHYSICS

Iron-55 from Nuclear Detonations

It is well known that the large numbers of neutrons released during nuclear bomb detonations (~0·5 kgm. neutrons per megaton explosion)[1] can induce high levels of radioactivities in the hardware and associated structures of the apparatus. Of particular interest is iron-55, an X-ray emitter with a half-life of 2·6 years, formed by $(n, 2n)$ and (n, γ) reactions on iron-56 and iron-54, respectively.

The site of high specific activity of iron-55 should be in the marine environment where stable iron exists in very low concentrations (~10 μgm./l. sea water). Radioactive iron falling on continental surfaces will be diluted readily in the large amounts of iron already existing there.

We thought it desirable to ascertain the present content of iron-55 in the oceans. Although this can be accomplished by measuring the vertical depth profiles of iron-55 contents at a number of marine locations, a more expeditious approach is the isolation of the iron activity from organisms that concentrate this element from the oceanic environment. The tuna fish which inhabit both the deep and shallow waters of the seas seemed especially suitable for this purpose since they may be considered to sample rather large areas because of their extensive migratory habits.

Ten kilograms of livers and hearts of tuna fish collected from the Pacific Ocean were digested with nitric and perchloric acids. The residue was dissolved in 8 *N* hydrochloric acid and the iron extracted with isopropyl ether. Further purification was achieved by fixing the iron on an anion exchange resin. The amount of elemental iron recovered was 2·5 gm.

The sample was assayed in a proportional counter shielded by 8 in. of iron and with an appropriate anti-coincidence arrangement. The purity of the activity was determined by comparing the spectrum of emitted X-rays with that of a standard iron-55 source. The specific activity of the samples, in February 1961, was found to be 82,000 d.p.m./gm. of iron. This value may be compared with those of other artificial and natural isotopes in the marine environment: strontium-90, 10 d.p.m./gm. strontium; cæsium-137, 500,000 d.p.m./gm. cæsium; and uranium-238, 700,000 d.p.m./gm. uranium. Since iron is generally the most abundant heavy metal in the marine biosphere, the activity-level of iron-55 is probably the highest. It should be noted that it is not great enough to constitute a health hazard.

An approximation of the iron-55 inventory in the Pacific may be obtained from the observed average specific activity of the oceanic iron. Assuming a concentration of 10^{-5} gm. iron/l. of sea water and an average oceanic depth of 4,000 m. (that is, 400 l./cm.^{2}), the iron-55 inventory in the Pacific should be of the order of $82{,}000 \times 10^{-5} \times 400$ or 330 d.p.m./cm.^{2} of oceanic area. Most of this activity probably still resides in the upper water inasmuch as the marine biosphere tends to promote an accumulation of iron in the oceanic mixed layer[2].

An attempt was made to find out whether any significant fraction of the iron-55 activity had already settled to the sea floor. The upper 1 cm. of sediment layer was taken from two cores of total cross-sectional area of 40 cm.^{2}, raised in the Pacific (28° 58′ N.; 117° 28′ W.; 3,600 m. depth) in April 1961. The sample was leached with 6 *N* hydrochloric acid and the iron in solution was extracted and purified as described above. The iron-55 activity in the entire sample was very small ($\leq$ 3 d.p.m.), implying that only an insignificant fraction of the activity had settled to the ocean bottom during the past few years.

In order to obtain a measure of atmospheric fall-out to the oceans, rain from San Diego was assayed for iron-55 activity. It yielded a value of 0·8 d.p.m./l. in February 1961. This indicates that the present contribution of atmospheric fall-out is small compared with the total inventory in the ocean. One metre of rainfall introduces only about 0·08 d.p.m. of iron-55 annually per cm.^{2} of oceanic area.

Acknowledgment is made to the Inter-American Tropical Tuna Commission for aid in obtaining the fish-livers. This work was sponsored by the Division of Biology and Medicine, U.S. Atomic Energy Commission.

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Quenching of Cation Vacancies in Doped Crystals of Sodium Chloride

As is well known, the thermodynamic defects developed by alkali halide crystals at high temperatures are predominantly of the Schottky type. The number of cation and anion vacancies in equilibrium with the crystal, at temperatures near the melting point, for example, may be as large as 10^{-4} of the total number of atoms. Attempts have, therefore, been made to quench these vacancies by very rapid cooling from these high temperatures, but without success, judging from the very similar values obtained for the electrical conductivities of the quenched and the annealed specimens[1]. (From the melt, however, some of the defects can be quenched.) This, however, is not surprising, since the relaxation times corresponding to the migration of vacancies, leading either to formation of pairs or to their exit from the crystal,

remain small, even at temperatures several hundred degrees below the melting point.

Cation vacancies can also be introduced, as is well known, by suitably doping the crystal. For example, by doping sodium chloride with cadmium chloride, it is easy to see that at sufficiently high temperatures the number of cation vacancies produced by the doping will be just the number of Cd^{++} ions held in solution. We find experimentally that such vacancies, unlike the natural Schottky ones, can be quenched effectively. There are many advantages, as we shall see presently, in working with such quenched crystals, since the number of cation holes is then known definitely, and can be made fairly large. It may be mentioned incidentally that though extensive work has been done on annealed specimens of doped crystals, by Maurer *et al.*[1], quenched specimens do not seem to have been studied.

In the present experiments large clear crystals of sodium chloride containing known molar concentrations c of cadmium chloride, ranging from 2×10^{-5} to 7×10^{-4}, were grown from the melt in the usual manner. They were annealed at about 1,050° K., which is 27° below the melting point of the pure crystal, and then suddenly dropped into liquid nitrogen and thus allowed to cool rapidly. Although many of the crystals developed cracks, it was possible to obtain specimens free from cracks that were large enough for electrical conductivity measurements. Fig. 1 gives a plot of the conductivity σ of the quenched crystals at four different temperatures up to about 720° K. These temperatures were low enough to leave the quenching undisturbed. The observed small residual conductivity of the 'pure' crystal at these temperatures was corrected for. It corresponded to a doping concentration of cadmium chloride of about $4{\cdot}6 \times 10^{-6}$. It will be seen that they plot into straight lines, naturally passing through the origin. In Fig. 2 are plotted the values of $\log(\sigma T/c)$ against $1/T$, from which it will be seen that the values for all the concentrations plot on the same straight

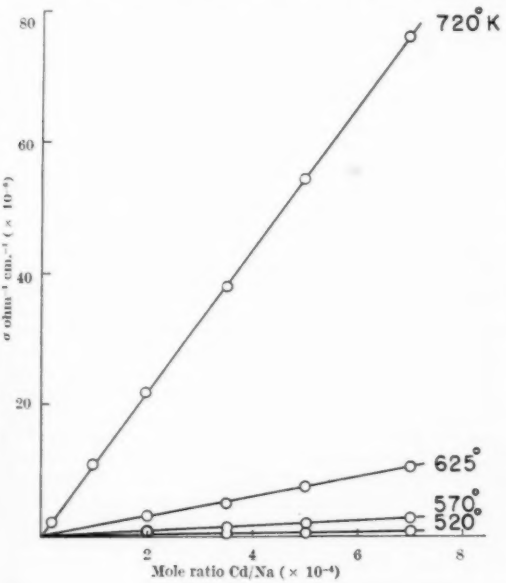

Fig. 1

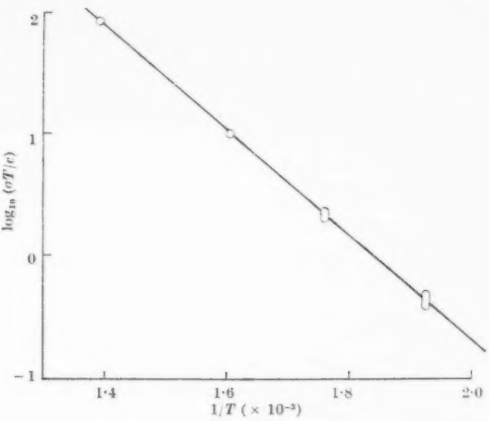

Fig. 2

line. One may draw the following conclusions from this: (1) that even at the highest temperature of measurement, namely, 720° K., the number of cation vacancies produced by quenching remains undisturbed, and is therefore independent of the temperature of measurement; (2) that this number is proportional to the concentration c up to the highest concentration studied, namely, 7×10^{-4}, and must, therefore, be the number obtaining at the high temperature, and hence the same as the number of Cd^{++} ions doped into the lattice; in other words, while all the cation vacancies produced by the dope at 1,050° K. have been frozen intact, almost none of the natural cation vacancies of the Schottky type, the number of which also is considerable at this high temperature, has been frozen; (3) since the relaxation time corresponding to the migration of a cation hole to the neighbourhood of Cd^{++} is small, that the observed proportionality of the quenched number of holes to the concentration of cadmium points to a low value for the energy of pairing of a cation vacancy with a Cd^{++} ion.

Using the well-known expression for the conductivity, namely, $\sigma = ne\mu$, where $\mu = (ea^2/kT)\exp(-U/kT)$ is the mobility of the cation vacancy, and $U = U_0 - \alpha T$ is the activation energy corresponding to the mobility, one obtains from the slope of the straight line in Fig. 2, $U_0 = 0{\cdot}85$ eV., and from the intercept on the y-axis the value $\exp(\alpha/k) = 460$, which is large, but of the right order of magnitude. This value of α would correspond to a temperature coefficient of U given by $-(1/U_0)\mathrm{d}U/\mathrm{d}T = 6 \times 10^{-4}$.

On annealing the crystal by very slow cooling from high temperatures, by about 15° in a day, the effective number of cation holes drops. This cannot be attributed to the formation of pairs of cation holes with Cd^{++} ions, since this process has the same small relaxation time as for the pairing of the Schottky vacancies, and would therefore have occurred even without the annealing, if the energy of combination had been large enough.

One is forced to the conclusion that the reduction in the number of cation holes by annealing must be due to the elimination of these holes, and of the same number of Cd^{++} ions, by a much slower process, probably by precipitation as cadmium chloride. This accounts for the possibility of quenching these cation vacancies, but not the Schottky vacancies. Such precipitation may be merely a conse-

quence of the decrease in the solubility of cadmium chloride with decrease of temperature. The development of turbidity by these crystals on annealing lends support to such a precipitation.

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Ultrasonic Velocity in Aqueous Solutions of Some Electrolytes

While carrying out investigations in a number of aqueous solutions of electrolytes, we have observed the interesting feature of a decrease of ultrasonic velocity with increase of concentration in the case of aqueous solutions of calcium iodide, zinc bromide, silver nitrate and cerous acetate, contrary to the normal type of behaviour exhibited in general by electrolytes. Velocity measurements in the above solutions of electrolytes at 30° C. were carried out using a fixed-path, variable frequency interferometer[1], at a frequency of 1·5 Mc./sec. The accuracy of the measurements is ± 1 m./sec. The variation of density with concentration in all the above cases is found to be linear.

The variation of ultrasonic velocity with concentration is shown in Fig. 1. In all the electrolytes investigated, zinc bromide, calcium iodide and silver nitrate exhibit a decrease in velocity of 88, 43 and 40 m./sec., respectively, for a concentration of 3 moles/litre. In spite of the peculiar behaviour in the variation of velocity with concentration, it is found that the adiabatic compressibility shows a regular decrease with increase of concentration in all these electrolytes. All other properties, like molar sound velocity and apparent molal compressibility, also showed a regular linear variation with concentration except in the case of cerous acetate, for which the variation of apparent molal compressibility with concentration is irregular.

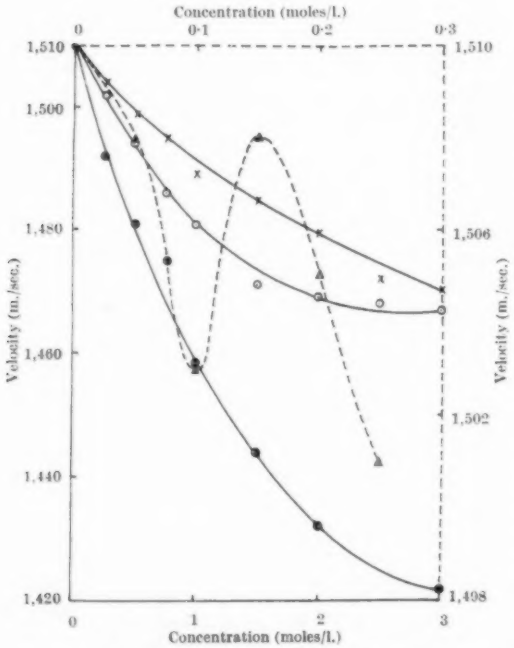

Fig. 1. Variation of ultrasonic velocity with concentration in aqueous solutions of electrolytes. ○, Calcium iodide; ×, silver nitrate; ⊗, zinc bromide; △, cerous acetate

It has been reported by earlier investigators[2–4] that electrolytes with heavy radicals or those having complex ions exhibit a decrease in velocity with increase in concentration. The decrease of ultrasonic velocity in the case of zinc bromide can be explained as due to the formation of complex ions, and that in calcium iodide as due to the heavy acid radical. But in the case of silver nitrate which does not form any complexes in aqueous solutions this result is unusual as similar salts like barium nitrate[5], with heavier metallic radicals, show an increase of velocity with concentration. In the case of cerous acetate the irregular variation of velocity with concentration is interesting.

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RADIATION CHEMISTRY

Photochemical Production of Semiconducting High Polymers

Polymers may be rendered semiconducting by heating to form graphitic structures[1]. Such non-specific treatment results in a drastic deterioration of physical properties of the polymer and a change in form of the samples. We have found that ultra-violet light irradiation of chlorinated high polymers transforms them into semiconductors. This treatment has advantages over the pyrolysis method in that: (*a*) by selective choice of ultra-violet wave-lengths specific chemical reactions are produced; (*b*) the reactions may be confined to specified areas. In fact, we have found that at the border of irradiated regions unique *p–n* junctions are produced.

In our experiments we irradiated films about 12μ thick of a commercially available saran which is a copolymer of vinylidene chloride (85 per cent) and vinyl chloride (15 per cent). All additives present were removed by soaking in acetone and then in ethanol so that no carbonyl groups are observed in the infra-red spectrum. For exclusively far ultra-violet irradiation we employed a 15-W. mercury resonance lamp (General Electrical Germicidal) which produces mainly 254 mμ. For near ultra-violet irradiation we used a 100-W. intermediate pressure lamp (Hanovia *SH*) in conjunction with a Corning 9–53 glass filter which removes all wave-lengths below 300 mμ, and for mixed irradiation this lamp was used without a filter.

Irradiation with far ultra-violet produces materials the spectra of which are given in Fig. 1. After subtracting out the background (curve *A*) the curves exhibit a broad maximum at about 285 mμ. The shape of the curve and the position of the maximum are maintained

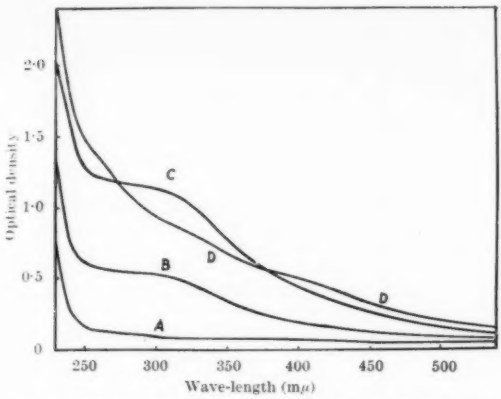

Fig. 1. Spectra of irradiated saran. *A*, original; *B*, far ultra-violet irradiated 2 hr.; *C*, far ultra-violet irradiated 4 hr.; *D*, sample *C* irradiated with near ultra-violet 1 hr.

as the irradiation is continued. This shows that distinct species are being produced in greater and greater amounts, but their relative concentrations are fixed. This is contrary to the generally adopted view[2] that increased irradiation of chlorinated polymers results in progressively more long-chain conjugation. The maximum at 285 mμ suggests the presence of a chlorinated compound containing three conjugated double bonds. It is possible that this is a ring compound formed between neighbouring chains. In fact, irradiation of saran by far ultra-violet is accompanied by cross-linking just as is the case with other high polymers[3], and the gel fraction (determined by insolubility in dioxane) is proportional to time of irradiation. Heating at 100° C. for a few hours of previously irradiated sample results in both an increase in absorption and an increase in cross-linking, both effects being enhanced the greater was the time of previous irradiation. Unirradiated samples heated in this manner show no cross-linking and no change in absorption. The absorption maximum at 285 mμ is proportional to the time of irradiation even up to large optical densities. It is evident that the sensitizer for this reaction is present in large concentration and since the original sample has a low absorption at 254 mμ this strongly suggests that the carbon–chlorine bond is the site of the photochemical reaction.

The tail of the absorption spectrum extends into the visible region and accounts for the brownish appearance of the irradiated film. Irradiated film exhibits a yellow-orange fluorescence (phosphorescence at liquid nitrogen temperature) under excitation with light up to about 400 mμ. Near ultra-violet light irradiation results in destruction of the species associated with the 285 mμ maximum by a photoxidation reaction and causes an increase in absorption below 240 mμ and above 390 mμ (curve *D*). Samples not previously irradiated with far ultra-violet are unaffected by near ultra-violet irradiation.

Far ultra-violet irradiation produces stable free radicals as shown by electron spin resonance spectra, and the spectral curves are symmetrical and structureless. For sample *B* of Fig. 1 there are 10^{15} spins/gm. The intensity of the signal is proportional to the time of irradiation and heating at 100° C. destroys the signal. Samples first irradiated with far ultra-violet and then with near ultra-violet show an unsymmetrical magnetic resonance absorption band. The free-radical species produced by far ultra-violet is preserved and the next species produced by near ultra-violet is superposed on the spectrum. Obviously the free radicals are not associated with the 285 mμ peak.

Samples irradiated with far ultra-violet show a considerable decrease in electrical resistance from that of the original saran sample (resistivity 10^{15} ohm cm.). Thus sample *C* of Fig. 1 has a thousand-fold smaller resistance than sample *A*. Subsequent irradiation with near ultra-violet influences the resistivity only slightly. The irradiated samples are photoconducting when excited by light below 300 mμ, and the photocurrent is proportional to the intensity of exciting light. Momentary illumination with the intermediate pressure lamp lowers the resistance to a hundredth that in the dark. Additional near ultra-violet irradiation increases the photoconductivity still further, while heating at 100° C. of previously irradiated samples lowers markedly the photoconductivity. It appears, therefore, that photoconductivity is associated with trapped unpaired electrons.

Interesting electrical effects are produced by irradiating two adjacent areas with the unfiltered intermediate pressure lamp for different lengths of time. For example, a portion of the film is irradiated for 5 min. and the whole sample is irradiated for 10 min. so that a sharp boundary is produced. Dusting with a mixture of charged powders (sulphur and red lead) reveals that negative charges lie mainly along a line in the less-irradiated region close to the boundary while positive charges lie mainly along a line close by in the more-irradiated region. The lines of opposite charge are separated by a thin neutral zone. This p–n junction exhibits rectification properties when illuminated with far ultra-violet, but does not do so in the dark. The presence of the neutral zone is also revealed photometrically. The samples show a colourless non-fluorescent line between adjoining areas, suggesting that a photochemical product migrates to the less-irradiated portion and reverses the effects of prior irradiation. An oxidation-reduction process might be the basis of this effect.

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CHEMISTRY

Effect of Chloride Concentration on the Aqueous Corrosion of a Magnesium Alloy

At each nuclear power station of the Central Electricity Generating Board, the spent-fuel elements will be stored under water for about one hundred days, by which time their radioactivity will have decayed sufficiently to permit them to be removed for reprocessing of the uranium fuel. The water in each cooling pond will be maintained at about *p*H 11·5 by adding caustic alkali to minimize corrosion of the magnesium alloy can (*A*12). Although corrosion should be slight, it is still, however, important, since the corrosion product is radioactive due to the presence of active isotopes developed under irradiation

from impurities in the magnesium alloy. In addition, accelerated corrosion can be expected on about 50 per cent of the cans in the pond due to carbon deposited on their surface from the carbon dioxide coolant during reactor operation. This type of accelerated corrosion is very sensitive to the presence of chloride ion in the water; the critical level for rapid attack has been estimated from corrosion tests as less than 10 p.p.m.[1]. Since all the power stations are sited near or on the coast, and most have uncovered cooling ponds, the possibility of enhanced corrosion, due to chloride pick-up, is very real, and an electro-chemical assessment of the effect of chloride and carbon on magnesium alloy corrosion is in progress.

The experimental method involves potentiostatic polarization runs from $-2{\cdot}0$ V. cathodic to $+2{\cdot}0$ V. anodic versus a saturated calomel electrode, at a rate of 0·17 V./min., the current and potential values being plotted simultaneously on an X–Y recorder.

Fig. 1 compares the result obtained using an annealed and pickled magnesium alloy specimen with that obtained from a specimen having a carbon deposit. The runs were carried out in a synthetic pond

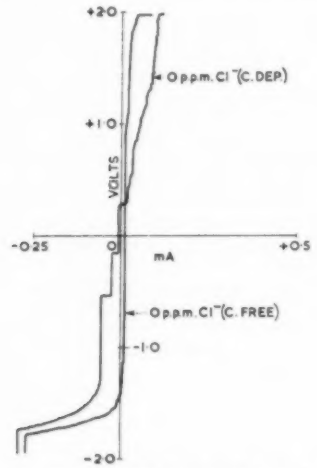

Fig. 1. A12 alloy. Effect of carbon-deposition

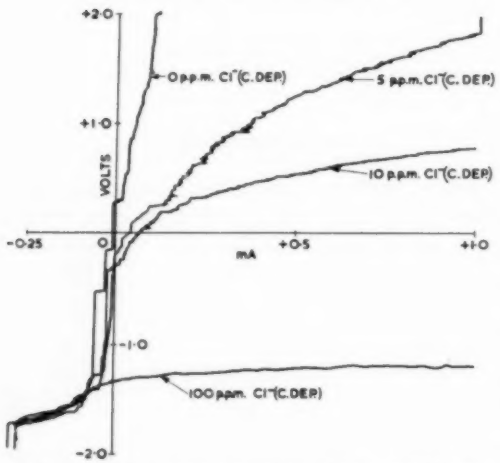

Fig. 2. A12 alloy with carbon-deposition. Effect of chloride-level

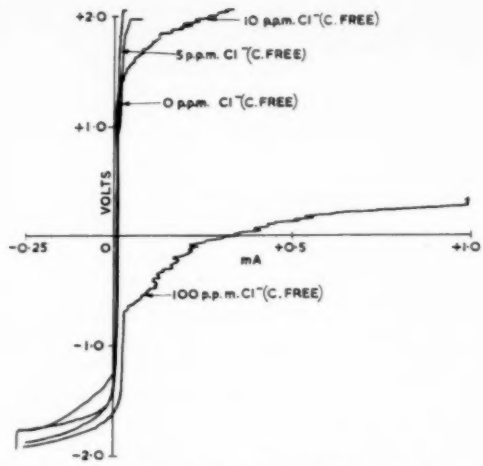

Fig. 3. A12 alloy annealed and pickled. Effect of chloride-level

water solution containing 200 p.p.m. sodium hydroxide and 530 p.p.m. sodium carbonate.

In Fig. 2, results are shown for a number of specimens with carbon deposition, in the same solution but at various levels of added chloride. These may be compared with the corresponding effect of chloride concentration on runs with annealed and pickled specimens in Fig. 3.

From these results it may be seen that while chloride exerts an effect on the behaviour of annealed and pickled magnesium alloy specimens at levels of 10–15 p.p.m. and above, the corresponding level for specimens with a carbon deposit is much lower, and is about 4 p.p.m.

We are grateful to Dr. J. S. Forrest, director of the Central Electricity Research Laboratories, Leatherhead, for permission to submit this communication.

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A New Inorganic Reaction: Nitric Acid–Hydrazoic Acid

Reaction of trivalent nitrogen with the azide ion, usually called the 'nitrite-azide reaction', is generally known and has been a subject of recent detailed investigations[1–4]. This azide reaction has been considered to be specific for trivalent nitrogen even in strongly acidic media, and the fact that certain reactions of pentavalent nitrogen (for example, oxidation processes of nitric acid, lead chamber process) are stopped by the addition of azide ions has been accepted as a proof that they proceed through the trivalent nitrogen[5].

Now it has been found that pentavalent nitrogen compounds react with hydrazoic acid in strongly acidic solutions. The reaction was studied polarographically, and the reaction products were analysed by means of gas chromatography. The experiments were performed with sodium nitrate and sodium azide added to the strongly acidic solution. The

course of the reaction can be followed polarographically either by means of the nitronium ion waves which appear in 15 *M* and more concentrated sulphuric acid[6], or by means of the wave of hydrazoic acid which is well developed in 8 *M* and more concentrated sulphuric acid solutions[7]. The stoichiometry of the reactants is nitric acid : hydrazoic acid = 1 : 2 and the products are a gaseous mixture of nitrogen, nitrous oxide and in some cases nitric oxide. The rate of the reaction is influenced markedly by the acidity of the reaction medium. When using sulphuric acid the following regions can be distinguished: with more than 13 *M* sulphuric acid the reaction proceeds so quickly that its time-course in the solution cannot be followed polarographically. In Fig. 1 it is seen that after addition of nitrate to the azide solution in sulphuric acid the azide wave with $E_{1/2} = 0{\cdot}7$ V. disappears and a new wave is formed with $E_{1/2} = -0{\cdot}9$ V. against a saturated mercurous sulphate electrode in 17 *M* sulphuric acid. This new wave is independent of time (cf. Fig. 1, curves 2 and 3) and can be easily removed by passing nitrogen through the solution (cf. curves 4 and 5). By further addition of nitrate, two waves are obtained which correspond to the depolarization effects of nitrites in the same medium[6] (Fig. 2). Below 13 *M* sulphuric acid the homogeneous kinetics of removing the hydrazoic acid from the solution can be followed after addition of nitrate (Fig. 3). The wave of hydrazoic acid is somewhat distorted after the addition of nitrate. This is due to the indistinct depolarization effects of nitrates in this medium which are manifested by shifting the hydrogen discharge towards the positive potentials and which are superimposed to the hydrazoic acid wave.

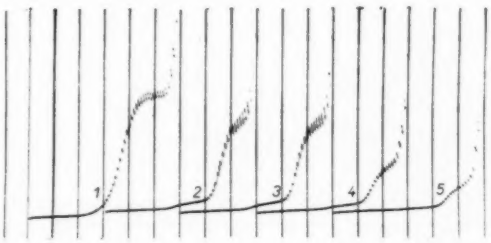

Fig. 1. Course of the hydrazoic acid–nitric acid reaction at acidities higher than 13 *M* sulphuric acid. 1, 10 ml. 14·5 *M* H_2SO_4 + 0·4 ml. 0·1 *M* NaN_3; 2, + 0·2 ml. 0·1 *M* $NaNO_2$; 3, curve 2 after standing for 10 min.; 4, nitrogen passed for 30 sec.; 5, nitrogen passed for another 1 min. $S = 1 : 40$; saturated mercurous sulphate electrode; from 0·0 V.; 200 mV./absc.

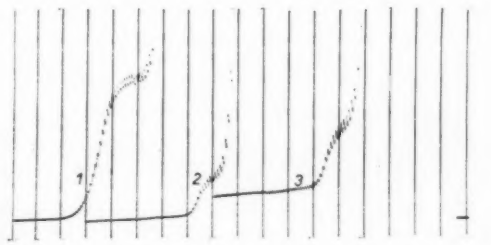

Fig. 2. Influence of nitrate on the product of hydrazoic acid–nitric acid reaction at acidities higher than 13 *M* sulphuric acid. 1, 10 ml. 14·5 *M* H_2SO_4 + 0·4 ml. 0·1 *M* NaN_3; 2, + 0·2 ml. 0·1 *M* $NaNO_2$; 3, + 0·1 ml. 0·1 *M* $NaNO_2$. $S = 1 : 40$; saturated mercurous sulphate electrode; from 0·0 V.; 200 mV./absc.

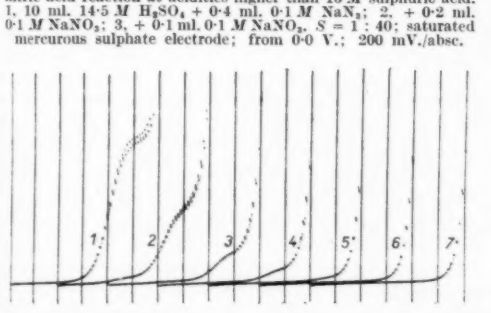

Fig. 3. Course of the hydrazoic acid–nitric acid reaction at acidities lower than 13 *M* sulphuric acid. 1, 10 ml. 13·0 *M* H_2SO_4 + 0·4 *M* NaN_3; 2–7, after addition of 0·2 ml. 0·1 *M* $NaNO_2$, the curves recorded at 3-min. intervals. $S = 1 : 40$; saturated mercurous sulphate electrode; from 0·0 V.; 200 mV./absc.

From the experimental results it follows that the reaction proceeds in three steps :

$$HN_3 + NO_2^+ \xrightarrow{k_1} 2NO + N_2 + H^+ \quad (1)$$

$$2NO + NO_2^+ + 2H^+ \xrightarrow{k_2} 3NO^+ + H_2O \quad (2)$$

$$3 \times (NO^+ + HN_3 \xrightarrow{k_3} N_2O + N_2 + H^+) \quad (3)$$

$$4HN_3 + 2NO_2^+ \longrightarrow 4N_2 + 3N_2O + H_2O + 2H^+ \quad (4)$$

Reaction (3), which is the known 'nitrite-azide' reaction, proceeds very rapidly even at low acidities and its rate increases with the acidity of the medium[1–4]. Hence it follows that in the strong acid medium which was used in the present experiments the rate of this reaction exceeds by far the rate of reactions (1) and (2). At acidities lower than 13 *M* sulphuric acid the rate-determining step is reaction (1), which is followed by rapid reactions (2) and (3) leading to the overall reaction (4). In the region above 13 *M* sulphuric acid the reaction (1) proceeds very quickly, $k_1 > k_2$, and the scheme is more complicated. From the 2 moles of nitronium ions (taking the stoichiometry according to (4) for $4HN_3 + 2NO_2^+ \ldots$) more than one mole can be consumed in the reaction (1) so that less than 1 mole of nitronium ions is left for reaction (2), and consequently a surplus of nitric oxide is formed which cannot be consumed in reaction (2). This is shown in Fig. 1, where the wave with $E_{1/2} = -0{\cdot}9$ V. corresponds to nitric oxide (as mentioned above the wave can be easily removed by bubbling nitrogen through the solution or by adding the nitrate, which leads to the formation of species of trivalent nitrogen according to (2) (Fig. 2). These conclusions in both concentration regions of sulphuric acid were confirmed quantitatively by gas-chromatographic analysis of the reaction products.

The full report of this work including further experiments will be published in *Collection of Czechoslovak Chemical Communications*.

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Spin Resonance and Electrical Conductivity of Indole-Iodine

Indole and iodine form a black complex that exhibits a strong electron spin resonance signal[1]. The interpretation that has been given is that indole, if complexed with a small acceptor such as iodine, may act as an especially strong electron donor due to the negative formal charge that exists on carbon 2 and 3.

A number of other iodine complexes also show strong spin resonance signals[2,3]. They also have a rather high electrical conductivity, at least for organic compounds. For the perylene and pyrene complexes, the spin resonance signal appears to be due to charge carriers[2]. On the other hand, this appears not to be true for the carotene complex[3]. Results presented here indicate that this is also not true for the indole-iodine complex, since there appears to be no correlation between the dependence on temperature of the spin resonance and that of the conductivity.

Spectra were taken on Varian V-4500 equipment modified to exhibit second derivatives[4]. The *R-F* power-level was approximately 3 mW., at which point the sample exhibited no saturation.

The spin resonance signal is slightly asymmetrical at low temperatures. It has a *g*-value of 2·0036 ± 0·0006. The line-width changes with temperature as indicated in Fig. 1. Since second derivatives were recorded, the number of free electrons was taken as proportional to $(\Delta H)^2 yT$, where y is the peak ordinate of the spectrum and T is the absolute temperature. The factor T is introduced to correct for the differences in distribution between the upper and lower spin states at different temperatures. The absolute number of unpaired spins was estimated by comparing the signal with a known amount of diphenylpicrylhydrazyl (DPPH).

Temperature was varied by running a stream of nitrogen at various temperatures past the sample. A thermocouple was inserted 1 in. from the sample and the assumption was made that the thermocouple measured the sample temperature when repeated runs showed no variation when taken over at least a 10-min. interval. In addition, equilibrium was checked by taking points on both increasing and decreasing temperature runs. The results (Fig. 2) indicate a lowering of the number of spins as the temperature is lowered.

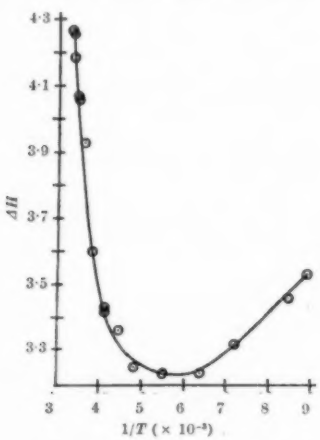

Fig. 1. Line-width in oersteds versus inverse temperature in inverse degrees K. The line-width is taken as the separation of the points of maximum slope of the absorption

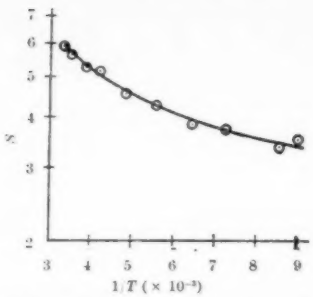

Fig. 2. Number of spins in 10^{18} spins/gm. versus inverse temperature

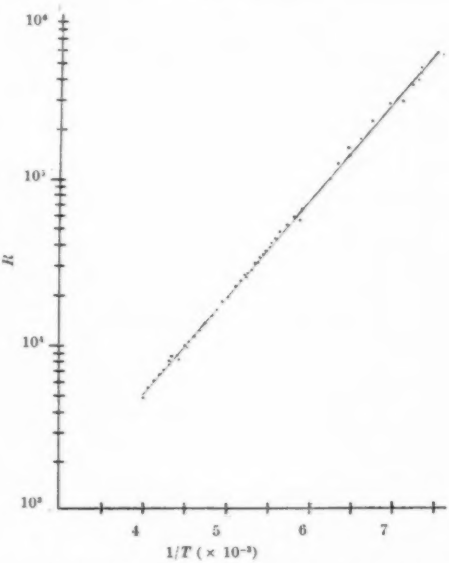

Fig. 3. Resistivity in ohm.-cm. versus inverse temperature

The electrical conductivity was measured on a sample that had been compressed between two brass screws that fit tightly into a 'Pyrex' tube of internal diameter 3·5 mm. Compression was applied and continued until changes in the electrical conductivity could be accounted for solely on the basis of changes in length of the sample. The brass screws served as electrodes, and the resistance was measured by a standard bridge arrangement. Results are shown in Fig. 3 and yield an activation energy of 0·11 eV.

A four-electrode method was also used and gave the same activation energy provided that the current density was sufficiently high. It was noted that below a current density of approximately 2×10^{-2} μamp.-mm.$^{-2}$ the resistivity was non-ohmic and followed a $V \sim i^{0.8}$ law.

It may be seen that no correlation appears between the spin resonance characteristics and those of the electrical conductivity. The spin resonance signal thus does not appear to be due to charge carriers. However, no interpretation of the signal is evident at present.

This work was supported by a grant from the Commonwealth Fund, grant No. *H*-2042(*C*3) of the National Heart Institute, a grant from the National Science Foundation, and grant No. 1 from the Quartermaster Research and Engineering Command, U.S. Army (Natick, Massachusetts). The work was done while one of us (B. S.) was a visiting investigator at the Institute for Muscle Research.

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Dependence on Temperature of the Distribution of Cations in Oxide Spinels

The fact that the cations in the typical spinel A_2BO_4 can be distributed in the so-called normal manner $A_2^{VI}B^{IV}O_4$, in the inverse manner $(AB)^{VI}A^{IV}O_4$, or 'randomly' with A and B ions statistically distributed over the sites, has long been used to characterize spinel structure phases. Verwey and Heilman[1], Gorter[2] and Romeign[3] have presented many data and lists on the 'type' of a particular spinel. Recent crystal field theory has also attempted to explain the position of a cation in oxide spinels[4]. Certain phases, for example $NiAl_2O_4$, have been characterized as partially inverse or even quantitatively as '85 per cent inverse', the cation distribution being $(Ni_{0\cdot15}Al_{0\cdot85})^{IV}(Ni_{0\cdot85}Al_{1\cdot15})^{VI}O_4$. There is in addition, of course, possible ordering on the octahedral and tetrahedral sites in phases such as $LiAl_5O_8$.

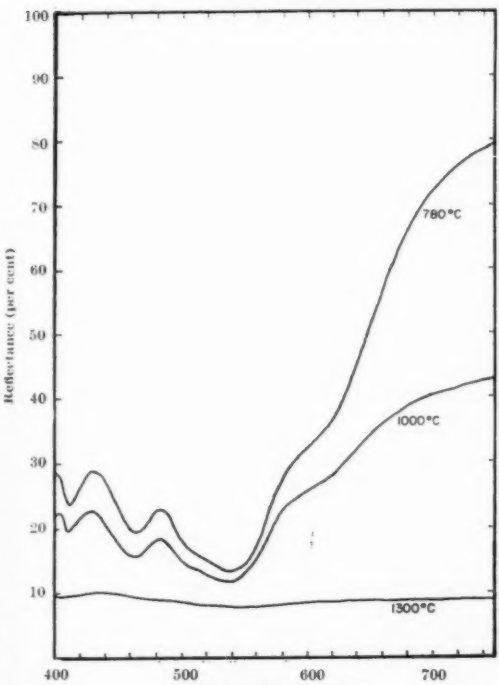

Fig. 1. Reflectance spectra of Co_2GeO_4 (with respect to MgO) samples. 780° C. sample prepared at 1,000 bars, then heated to 1,170° and 1,300° C.

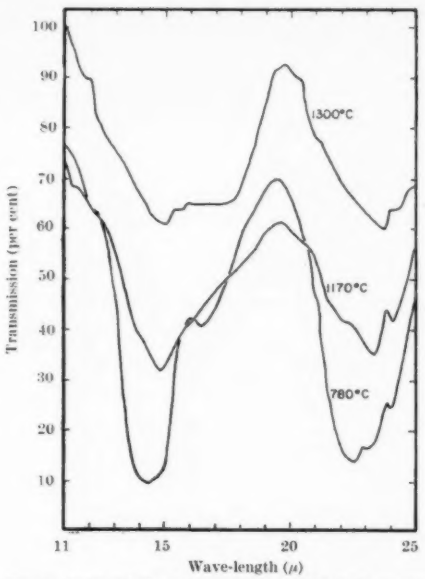

Fig. 2. Infra-red absorption patterns of Co_2GeO_4 samples after different heat treatments

It is the purpose of this communication to direct attention to the fact that in an extensive series of experiments it has been shown that in the majority of spinels, including very common ones, the cation distribution is a function of the temperature and pressure of formation. The following phases have been prepared and equilibrated in the temperature-range 400–1,800° C. and pressure-range 1–100,000 atm.: Ni_2GeO_4, Co_2GeO_4, $NiAl_2O_4$, $MgAl_2O_4$, Zn_2TiO_4, Co_2TiO_4, 'Ni_2TiO_4', $CdGa_2O_4$, $ZnGa_2O_4$, $NiCa_2O_4$, $LiAl_5O_8$, etc.

The change of the cation distribution has been characterized by the correlation of four properties: unit cell dimensions, X-ray intensities, reflectance spectra in the visible region and infra-red absorption spectra from 11–25μ. Figs. 1 and 2 compare the lowest temperature, high-pressure form of Co_2GeO_4 with the same sample at higher temperatures at 1 atm. There is a very substantial change from a nearly perfect inverse state at low temperature to a substantially normal state at high temperature. The actual assignments of different infra-red absorption bands to various modes involving cation-anion dipoles with the cations changing co-ordination has been shown to be a complex but useful problem[5]. Substantial changes were found for each of the phases mentioned.

Mutual crystalline solubilities of many spinels have also been studied as a means of showing the cation distribution. Different degrees of deviation from ideality at different temperatures (for the same system) indicate the relation between the temperature and cation distribution in spinels. The mutual

crystalline solubility relations between Ni_2TiO_4–Zn_2TiO_4 can likewise be explained by the temperature dependence of the degree of 'normalcy' of the 'Ni_2TiO_4', which does not form a compound at all. At higher and higher temperatures more and more nickel enters the tetrahedral sites, permitting 'nearly pure' Ni_2TiO_4 to be prepared in normal structure.

There is still some question as to whether or not all these reactions are reversible and whether we have true second-order phase changes over a wide temperature-range, with the degree of order determined by the temperature.

It is clear from these results that no spinel can be adequately characterized by reference to a table since the temperature and pressure of its formation determine (either stably or metastably) the cation distribution. The results also point to the necessity of presenting temperature-dependent functions for each ion as an essential refinement in crystal field approaches to the problem[4].

This work forms part of the studies in crystal chemistry supported by the U.S. Army Signal Corps under Contract No. *DA–36–039–SC–85311*.

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Reaction of Ethane with a Clean Rhodium Film

I have observed that when ethane at low pressure ($1{\cdot}2 \times 10^{-3}$ torr) comes in contact with a 27° C. rhodium film which was prepared in ultra-high vacuum (1×10^{-9} torr), it will decompose to yield gaseous methane and an adsorbed residue with a composition of $CH_{1.5}$. Others[1,2] have observed this cracking reaction to occur at temperatures above 160° C. on metal surfaces of unknown cleanliness.

The rhodium film was prepared in ultra-high vacuum by pulse evaporating[3] a small coil of 0·005 in. diameter, 99·99 per cent pure rhodium wire in the centre of a 4 in. diameter spherical 'Pyrex' glass reaction vessel which had been baked at 450° C. for 18 hr. The reaction vessel was held at 100° C. during the film deposition. The average film thickness, measured at the completion of an experiment by X-ray emission spectroscopy, was found to be about 30 Å. in most cases. The volume of the system was about 900 c.c.

After the film was deposited, the reaction vessel was isolated from the pumping system by closing a metal valve (Granville-Phillips Co., Pullman, Washington). Five minutes were allowed for the 0·090-in. diameter filament leads to cool below 100° C. Mass spectrometrically pure ethane was admitted through a glass break-seal. The pressure in the reaction vessel was followed by a thermocouple gauge which was calibrated against a McLeod gauge for each gas present and for mixtures thereof. At various intervals, the reaction was quenched by freezing out the ethane in a cold finger with liquid nitrogen and the residual pressure measured.

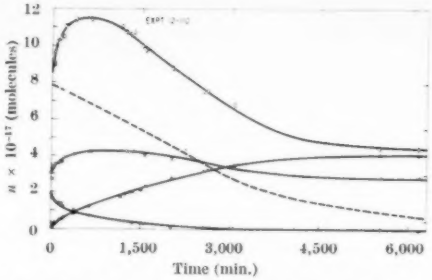

Fig. 1. Rate of decomposition of ethane on a 27° C. rhodium surface and the rate of product formation. ○, nC_2H_6; △, nCH_4; □, nC; ◇, nH. – – –, H/C (surface)

When the liquid nitrogen non-condensable pressure attained a constant value, it was assumed that the reaction had ceased. At this point the gases in the reaction flask were removed and analysed with a mass spectrometer. In all cases only methane was found.

These experiments were also carried out at a film temperature of 100° C. The films used at 100° C. were deposited on a 100° C. glass substrate.

A blank experiment was performed in which ethane was admitted to a baked-out reaction vessel at 100° C. There was no decomposition after 47 contact hr.

The results from a typical experiment carried out on a rhodium film with an average thickness of 30 Å. at 27° C. are shown in Fig. 1. The number of carbon atoms and hydrogen atoms present on the film at time t were calculated from the initial amount of ethane and the amount of ethane and methane present at time t as determined from the thermocouple gauge readings.

Plotting the log of the ethane pressure against time yielded a straight line. Thus, the disappearance of ethane obeys the first-order rate equation:

$$dn_{C_2H_6}/dt = -kn_{C_2H_6}$$

with an initial rate constant of $2{\cdot}13 \times 10^{-3}$ min.$^{-1}$. The rate of disappearance of ethane on a rhodium film at 100° C. also obeyed a first-order equation with a rate constant of about 0·5 min.$^{-1}$. The temperature dependence of the initial rate constant gives an Arrhenius activation energy of 16 ± 1 kcal./mole for the disappearance of ethane. The (H/C) surface ratio is about 1·5 at the end of the reaction for both film temperatures. When deuterium was added, the residue desorbed from the rhodium film as deuterated methane, indicating the presence of only monocarbon fragments on the surface.

The addition of oxygen to a clean rhodium film at 100° C. was found to inhibit the subsequent decomposition of ethane. The rate of disappearance of ethane was decreased by a factor of ten over that for a clean film at 100° C.

These experiments show that ethane will crack on a clean rhodium surface at room temperature. The rate of reaction is a sensitive function of both the surface temperature and surface cleanliness.

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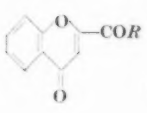
Some New Esters of Chromone-2-Carboxylic Acid

WITH the view of further pharmacological work by one of us (K. V.-B.), it was decided to synthesize certain esters of chromone-2-carboxylic acid, not previously described[1-4]. The following compounds were obtained: dimethylaminoethyl-chromone-2-carboxylate hydrochloride (I*a*), the methiodide of the base (I*b*); tropyl-chromone-2-carboxylate (II*a*), its hydrochloride (II*b*), methobromide (II*c*) and methiodide (II*d*):



(I*a*) R=O.$CH_2CH_2NMe_2$.HCl

(I*b*) R=O.$CH_2CH_2NMe_2$.IMe

(II*a*) R=O.$C_8H_{11}N$=O.CH(CH$_2$—CH=CH—CH$_2$)N.Me

(II*b*) R=O.$C_8H_{14}N$.HCl ; (II*c*) R=O.$C_8H_{14}N$.BrMe ;
(II*d*) R=O.$C_8H_{14}N$.IMe ; (III*a*) R=OH ; (III*b*) R=Cl

Chromone-2-carboxylic acid (III*a*), white needles from alcohol–water, melting point 256–259° (decomposes) (lit. (1), m.p. 256–263° (decomp.)), and its chloride (III*b*), white needles from petroleum ether (50–65°), m.p. 105–106° (lit. (3), m.p. 105·5–106·5°) (found: Cl, 16·6. Calc. for $C_{10}H_5O_3Cl$: Cl, 17·0) were synthesized following the technique of Zagorevskii *et al.*[3].

(I*a*) was obtained by treating 1·5 moles of (III*b*) with 1 mole of 2-dimethylaminoethanol, for 4·5 hr. in toluene by refluxing; white needles from methanol, m.p. 202–204° (decomp.); R_F: *n*-butanol/acetic acid/water (6 : 1 : 2; v/v) (Solvent *A*), 0·35; *n*-butanol/2 *N*-hydrochloric acid (1 : 1; v/v; upper phase) (Solvent *B*), 0·70 (found: C, 56·4; H, 5·5; Cl, 12·0; N, 4·8. $C_{14}H_{15}NO_4$.HCl requires C, 56·5; H, 5·4; Cl, 11·9; N, 4·7).

(I*b*) was obtained by treating 1·5 moles of (III*b*) with 1 mole of choline chloride, for 8 hr. in toluene by refluxing. The crude methochloride was transformed into methiodide by adding sodium iodide in methanol; white plates from methanol, m.p. 224–226° (decomp.); R_F: Solvent *A*, 0·59 and *B*, 0·56 (found: C, 44·4; H, 4·7; I, 31·0; N, 3·4. $C_{15}H_{18}INO_4$ requires C, 44·7; H, 4·5; I, 31·5; N, 3·5).

(II*a*) was obtained by treating 1·5 moles of (III*b*) with 1 mole of tropine in pyridine, left overnight at room temperature, poured into dilute potassium carbonate and extracted with chloroform; white prisms from petroleum ether (50–55°), m.p. 107–109°; R_F: Solvent *A*, 0·68 and *B*, 0·72 (found: C, 68·8; H, 6·0; N, 4·4; $C_{18}H_{19}NO_4$ requires C, 69·0; H, 6·1; N, 4·5).

(II*b*) was obtained by bubbling dry hydrogen chloride into a solution of (II*a*) in ether; white needles from methanol-ether, m.p. 247–251° (decomp.); R_F: Solvent *A*, 0·68 and *B*, 0·73 (found: C, 58·8; H, 6·2; Cl, 9·4; N, 4·0. $C_{18}H_{19}NO_4$.HCl.H_2O requires C, 58·8; H, 6·0; Cl, 9·6; N, 3·8).

(II*c*) was obtained from (II*a*) dissolved in acetone, by adding methyl bromide at room temperature; white plates from methanol-ether, m.p. 270–271° (decomp.); R_F: Solvent *A*, 0·60 and *B*, 0·60 (found: C, 53·6; H, 5·8; Br, 19·0; N, 3·4. $C_{19}H_{22}BrNO_4.H_2O$ requires C, 53·5; H, 5·7; Br, 18·7; N, 3·3).

(II*d*) was obtained from (II*a*) in a similar way to (II*c*), but using methyl iodide; white plates from aqueous ethanol, m.p. 276–277° (decomp.); R_F: Solvent *A*, 0·60 and *B*, 0·60 (found: C, 49·8; H, 5·0; I, 27·7; N, 3·0. $C_{19}H_{22}INO_4$ requires C, 50·1; H, 4·9; I, 27·9; N, 3·1).

The melting points were determined on a Kofler hot stage (Leitz, *M*350). The chromatograms were run on Whatman No. 1 paper by ascending technique, at 24°. The spots were developed with Draggendorff's reagent[5].

We are grateful to Prof. Mario R. Falco for advice and encouragement during this research.

Note added in proof: We have now obtained the original work of Zagorevskii *et al.* (*J. Gen. Chem. U.S.S.R.*, **29**, 2267; 1959) in which the ester (I*a*) is described. This compound is not mentioned in *Chem. Abs.*, **54**, 11007 (1960).

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BIOCHEMISTRY

Structure of Feather Keratin

FEATHER keratin is the outstanding protein fibre structure that still lacks an interpretation generally acceptable in any detail. It has long been known[1] that its X-ray diffraction diagram is of a β-type with the strong indication that the residue-length is only about $3\frac{1}{10}$ Å. instead of the $3\frac{1}{3}$ Å. and $3\frac{1}{2}$ Å. found in β-keratin and silk fibroin, and it has corpuscular properties besides[2], especially as demonstrated in the paper by Bear and Rugo[3], supported also by physico-chemical studies and the finding that the unit is probably a cyclic polypeptide[4]. In Bear and Rugo's scheme the structure is based on a net of corpuscular units the nodes of which are at approximately (0, 0), (0, $\frac{1}{2}$), ($\frac{1}{2}$, $\frac{1}{4}$), and ($\frac{1}{2}$, $\frac{3}{4}$), the plane of the net containing both the main-chain and side-chain directions. Continuing from this point and associated observations by the same authors, it is the purpose of this communication to state what we believe to be a very strong case for the structure of the corpuscles themselves. The principal steps in the argument may be summarized as follows :

(1) There are three polypeptide β-configurations ('pleated sheets')[5] with interchain CO . . . NH hydrogen bonds that are linear (see, for example, ref. 6 for our own style of models illustrating this), and in these the amino-acid residue-lengths are approximately $3\frac{1}{2}$ Å., $3\frac{1}{3}$ Å., and $3\frac{1}{10}$ Å., respectively. In the first two the chains are anti-parallel and dyad screws, respectively, and they satisfy the requirements of silk fibroin and β-keratin. In the third the chains are 'parallel polar' ; that is, they run in the same direction but are not screws, each chain having all its CO groups on one side and all its NH groups on the other side. It is a fair hypothesis that

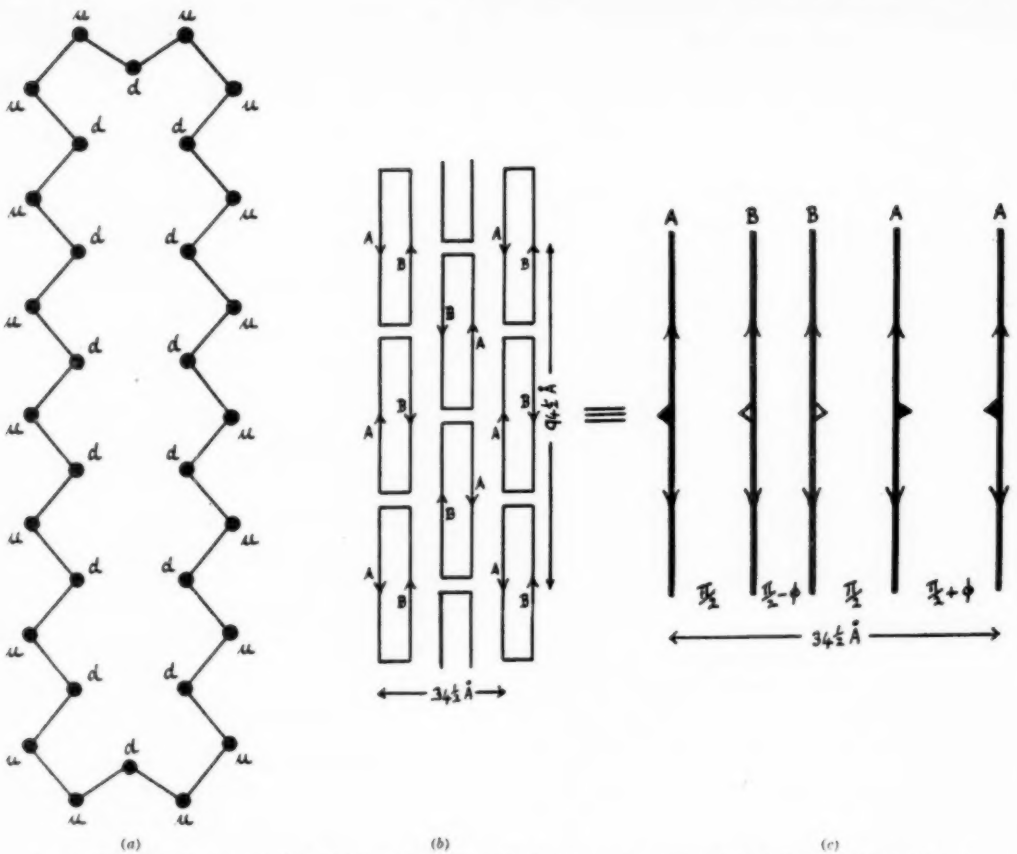

Fig. 1. Diagrammatic representation of (*a*) the proposed 32-residue loop-corpuscle, α-carbons being indicated by black dots and the bonds to β-carbons by *u* (up) and *d* (down); (*b*) the arrangement of polypeptide chains in conformity with the net pattern of Bear and Rugo; and (*c*) the equivalent scheme of chains for the purpose of deriving the equatorial intensities

this third arrangement, with its residue-length of about $3\frac{1}{10}$ Å., is the basis of the third known natural β-structure, that of feather keratin[6,7].

(2) The inference receives immediate strong support from the striking contrast between the meridional reflexions of fibroin and β-keratin on one hand and of feather keratin on the other. In relation to the axial sub-unit of a pair of consecutive residues, the former show no 1st order but a strong 2nd order (and also a 6th), while the latter shows a strong 1st order but no 2nd order (and no 6th either).

(3) Feather keratin contains a high proportion of proline residues (about one-tenth)[8], and these fit readily into the parallel-polar configuration[9], but not without strain into the other two β-configurations. We suggest that it is indeed the proline residues which impress the parallel-polar configuration on the feather keratin structure.

(4) Apparently the only other chain-configuration which has a residue-length of about 3·1 Å. and which can also incorporate proline without strain is the trigonal helix that is the basis of collagen[3]. A collagen-type structure for feather keratin has in fact been advocated by Ramachandran[10], but in our view it is quite ruled out by the infra-red data. Collagen has a unique, 'diagnostic' NH-stretching frequency at 3,330 cm.$^{-1}$ as compared with the 3,300 cm.$^{-1}$ found with the other fibrous proteins including feather keratin, and its other CO and NH frequencies are also above the range found with accepted β-structures and feather keratin[11].

(5) In conformity with Bear and Rugo's Fig. 6, we propose the loop-corpuscle structure represented diagrammatically, and most briefly for the present, by Fig. 1, for which we have constructed satisfactory scale-models both of our own and of the Courtaulds type. There are 32 residues per loop, and two features to be specially noted are: (1) that, looking transversely, the β-carbons on opposite sides are in register as well as the α-carbons; (2) that the CO groups all point upwards along one side but downwards along the opposite side, thus cancelling out the polar imbalance and any bending tendency that might otherwise accrue.

(6) Bear and Rugo's net in any event accounts most plausibly for the dominant meridional reflexion at 23·6 Å., and now our own calculations show also that, on the basis of Fig. 1*a* and the chemical-analytical[8] average residue-weight of 98·5, the reflexion near 6·2 Å., corresponding to the 'first order' of the characteristic residue-pair, should be about 150 times as strong as the 'second and sixth orders' near 3·1 Å. and 1·03 Å., respectively. (The observed intensities are strong : 0 : 0.)

(7) The equatorial reflexions are the first four orders of about 34½ Å., plus a fuzzy reflexion apparently corresponding to the CO . . . NH linkage transverse to the loop. The former change with the water-content and various swelling pre-treatments[3], and they have still to be worked out in detail; but preliminary calculations based on effectively four chains of appropriately different weights and separations, as in Fig. 1c, are already in sufficiently good agreement to indicate that this is probably the correct approach.

(8) Bear and Rugo's requirement of a plane-net structure, and the vagueness of the CO . . . NH linkage, are explained at once by our models, which show that continued regular superposition of loops will in general be obstructed by the proline residues. With one special arrangement of the latter, though, correct 'sandwich-pairs' are possible, but nothing thicker, suggesting a limiting corpuscle-weight of perhaps about 6,400 as compared with Woodin's[4] physico-chemical estimate of about 10,000.

(9) Finally, the calculated density of our structure is 1·32, compared with the observed value of 1·27 (ref. 12). The difference is in the right direction for a high polymer and is quite unobjectionably small.

Two other proposed structures for feather keratin are those of Krimm and Schor[13] and of Fraser and MacRae[14]; but we believe that the one outlined here offers a better interpretation of the experimental facts, as we hope to show more fully in a later publication.

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Simultaneous Determination of Dextran and Glucose in Serum

In the course of an investigation on the mechanism of 'anaphylactoid' reaction in rats, the need was felt for an accurate and simple procedure for the simultaneous determination of dextran and glucose in small amounts of serum. For this purpose the following method was developed. It utilizes the *o*-toluidin – glucose coupling reaction[1], producing a green colour suitable for colorimetric measurement. Dextran is precipitated from the serum by the *o*-toluidin – acetic acid reagent followed by hydrolysis and the colour reaction. Serum glucose is directly determined from the supernatant layer of the dextran precipitate.

Blow 0·1 ml. serum, containing 25–400 μgm. dextran, into 10-ml. glass-stoppered test-tubes containing 4 ml. of *o*-toluidin reagent (6 per cent *o*-toluidin in glacial acetic acid prepared immediately before use). Put the tubes in a refrigerator at 4° C. for 30 min. Centrifuge (3,500 r.p.m.) for 30 min. Decant the supernatant carefully into another test-tube. Place the latter in a boiling water-bath for exactly 8 min. Cool and read the optical density in a spectrophotometer or filter photometer at or near 625 mμ. A standard glucose solution is treated in the same way as serum.

The tubes containing the precipitated dextran are dried by placing them in an inverted position on a towel. 0·2 ml. of 4 *N* sulphuric acid is added and the precipitate dissolved by shaking. The tubes are loosely stoppered with glass stoppers (secured by a rubber band) and placed in a boiling water-bath for 75 min. After cooling, 8 ml. of *o*-toluidin reagent is added and the contents mixed. After boiling for exactly 8 min. the colour intensity is determined as above. A standard solution of dextran or glucose is carried through the entire procedure. In the latter case the result is multiplied by 0·9 (ref. 2), since 100 mgm. dextran is equivalent to 111 mgm. glucose.

The efficiency of dextran precipitation by the reagent was checked as follows: 0·1 ml. portions of 200, 400, 600, 800 mgm. dextran/100 ml. solutions were pipetted into 4 ml. of 6 per cent *o*-toluidin in acetic acid. After cooling and centrifugation, 20 ml. of 96 per cent alcohol was added to 2 ml. of the supernatant to precipitate the part of the dextran possibly remaining soluble in the reagent. The tubes were kept in the refrigerator overnight and, after centrifugation and decantation of supernatant, an anthrone reaction[2] was carried out with the residue. Sixteen determinations were made, optical densities being read in a Beckman *DU* spectrophotometer. In the range of 200–600 mgm./100 ml., the supernatant contained no anthrone-positive material which could be precipitated with alcohol. The supernatant contained only in the case of the 800 mgm. dextran/100 ml. an average of 1·37 per cent of total dextran.

The adequacy of the hydrolysis of dextran as used by Dimler *et al.*[2] was also investigated. 10, 50, 75, 100, 150 and 200 mgm. dextran/100 ml. solutions in 4 *N* sulphuric acid were kept in a water-bath at 100° C. for 75 min. After cooling, 8 ml. of 96 per cent alcohol were added to 0·8 ml. of hydrolysate, the tubes were kept at 4° C. overnight, centrifuged, the residue was washed once with 96 per cent alcohol and the anthrone reaction carried out. Six triplicate determinations were carried out. Traces of anthrone-positive material were found in 3 tubes, but not in the parallel tubes;

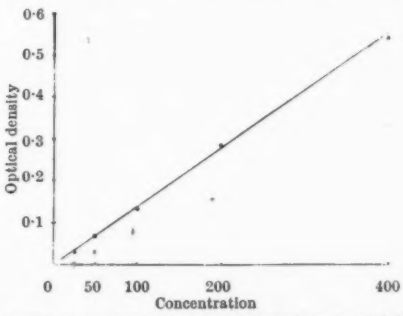

Fig. 1. Colour reactions of dextran solutions in water after hydrolysis. Abscissa: concentration of solutions. Ordinate: optical density determined at 625 mμ in 1-cm. cells in the Beckman *DU* spectrophotometer. Each point is the average of three determinations

therefore extraneous carbohydrate contamination was assumed in these cases.

In Fig. 1 is shown the colour reaction of dextran solutions in water after hydrolysis. There is good agreement with Beer's law within the concentration-range 25–400 mgm./100 ml. Dilution of serum is recommended for higher concentrations.

The coefficient of variation (per cent standard deviation) obtained by 20 parallel determinations on rat serum was 2·7 per cent for glucose at a level of 194 mgm./100 ml. and 2·5 per cent for dextran at a level of 184 mgm./100 ml.

We are indebted to Glaxo Laboratories, Ltd., for a gift of 'Clinical Dextran' used in this work. Dr. G. Szepessy, of the Central Research Laboratory of this University, kindly determined the optical densities.

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Emulsifying Capacity of Blood Sera

In view of the suspected link between fat metabolism and atherosclerotic disease, the state and behaviour of the emulsified fats in the blood serum have been the object of numerous investigations. Thus, for example, stabilized emulsions of lipoproteins, of coconut oil, and of olive oil have been added to sera in order to demonstrate the clearing reaction of heparin[1]. It seems, however, that the ability of different sera to emulsify various fats has never been investigated systematically.

In order to test the emulsifying ability of different sera, a simple, reproducible turbidimetric method of measurement has been developed. Briefly, the serum is shaken under controlled conditions with 0·5 per cent olive oil. The optical extinction rises to a maximum with time and then begins to fall; at 37° C. the maximum extinction is reached in about 20 min. The maximum extinction value we termed the 'emulsifying capacity' of the serum. The dispersion of 0·5 per cent olive oil in 0·005 per cent 'Lissapol' (run parallel with the experiments) served as control.

The emulsifying ability of a given serum has been found to be reproducible to within about ± 2 per cent.

Microscopic investigation showed that greater turbidity corresponds to a smaller average oil-droplet diameter.

After the reproducibility of the results was proved beyond doubt, studies have been initiated of the influence of various factors on the maximum turbidity obtained. The following is a summary of the results:

(1) There is an optimum shaking rate of about 200 beats/min. for the development of high turbidity emulsions.

(2) At oil concentrations between 0·1 and 1 per cent the maximum turbidity has been found to be proportional to the logarithm of the oil concentration.

(3) The emulsifying ability of sera from different animals has been found to vary both from animal to animal, as well as from species to species. Thus, sheep and cattle sera show higher emulsifying ability than pig sera; this variation in emulsifying capacity is well outside the variations found with sera from individual animals of the same species.

(4) The type of oil used determines, to a large extent, the maximum turbidity. In general, oils yielding high-turbidity emulsions have high acetyl and iodine numbers, and low viscosity. It must be stressed, however, that these differences are substantially smaller when the experiments are conducted at 37° C., although some differences still persist; thus, coconut oil yields substantially coarser emulsions both at room temperature and at 37° C.

(5) The addition of surface-active compounds exerts a strong influence on the maximum turbidity obtained. I found that in the presence of small concentrations (5×10^{-4} *M*) of surfactive acids (for example, oleic acid) the emulsification decreases, while the addition of the same amount of surfactive bases (for example, long-chain amines) or of neutral surfactants (for example, cetyl alcohol) increases the maximum turbidity. The effect of the surfactant is the same, whether it is added to the serum or to the oil phase.

(6) Diluting the serum up to ten-fold with isotonic phosphate buffer (*p*H 7·2) increases the maximum obtainable turbidity; further dilution results in a decrease of the maximum turbidity. The reasons for this particular behaviour remain to be investigated; they could be attributed tentatively to either changes in the protein surface and/or to viscosity changes.

Examination of some of the physico-chemical properties of the resulting emulsions have shown that:

(1) The electrophoretic mobilities of the oil droplets seemed unchanged by the nature of the surfactant added, a result which warrants further investigation.

(2) The interfacial surface tension is only slightly lowered in the presence of surface-active compounds; the degree of emulsification obtained seems unrelated to the interfacial surface tension.

Clinical investigations are at present being carried out in the Lidcombe State Hospital, Sydney. Although it is too early at present to draw conclusions from the results so far obtained, it is already evident that there are individual variations in the emulsifying abilities of human sera; sera obtained at different times from one and the same person can also show marked variations. Thus, the emulsifying ability obtained with the serum of a given individual invariably decreases after the ingestion of fats. Interesting effects have been observed on intravenous injection of heparin; they will form the subject of a separate communication, as will the details of the work presented here.

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Hydrolysis of Fatty Acid Esters of Riboflavin by Pancreatic Lipase

Fatty acid esters of riboflavin, fat-soluble riboflavin derivatives, have been synthesized in our laboratory to widen the application of riboflavin to pharmaceutical and nutritional fields[1–3]. As a fundamental problem of this application, the possibility of enzymic hydrolysis of these esters to riboflavin and fatty acids has to be investigated first. This communi-

cation deals briefly with their hydrolysis *in vitro* by pancreatic lipase.

Fatty acid esters were synthesized by esterification of riboflavin with fatty acid chloride, and riboflavin 2′, 3′, 4′, 5′-tetrabutyrate, tetracaprate, and tetrapalmitate were obtained as reported before[1-3]. Pancreatic lipase was supplied by the Nutritional Biochemical Corporation. The activity of the enzyme preparation was checked by using tributyrin and 'Tween 20' as substrate. The reaction mixture consisted of 0·5 ml. of 4 mmole/l. fatty acid ester of riboflavin suspended in 1 per cent polyvinyl alcohol (degree of polymerization: 500), 0·5 ml. of enzyme solution (20 mgm. protein/ml.) and 1·0 ml. of McIlvaine buffer (*p*H 7·3).

The reaction mixture was incubated at 37° C. for 2 hr. with gentle shaking. After incubation, the test solution was analysed by paper chromatography using an upper layer of *n*-butanol/acetic acid/water (4 : 1 : 5, v/v) as a mobile phase. After 10 hr. development at 20° C., the paper was removed and allowed to dry at room temperature in the dark. The paper strip was examined under ultra-violet light, and flavins were detected by their yellow fluorescence.

When riboflavin tetrabutyrate was incubated with pancreatic lipase, yellow fluorescent spots were observed on the paper at R_F values of 0·3, 0·5, 0·6, 0·7 and 0·97. Substances at R_F value 0·3 and 0·97 were identified with riboflavin and riboflavin tetrabutyrate respectively. Substances at R_F values 0·5, 0·6 and 0·7 were supposed to be the intermediate compounds from riboflavin tetrabutyrate to riboflavin. The riboflavin part was eluted with hot water, and the other yellow fluorescent spots were eluted with 98 per cent ethyl alcohol. The amount of flavin in both eluates was measured by the lumiflavin fluorescence method[4]. The enzyme activity was expressed by the amount of free riboflavin split off from fatty acid ester of riboflavin.

Riboflavin tetracaprate and tetrapalmitate were also examined in the same way, and hydrolyses of these substances by pancreatic lipase were compared with each other. The results are shown in Table 1. Among fatty acid esters of riboflavin tested, tetrabutyrate was found to be most easily hydrolysed, followed by tetracaprate and tetrapalmitate. In the case of tetrabutyrate, optimum *p*H and temperature of the enzyme were found at 7·3 and 37° C. respectively.

Table 1. Comparison of Hydrolysis of Fatty Acid Esters of Riboflavin by Pancreatic Lipase

Substrate	Rate of hydrolysis (per cent)
Riboflavin tetrabutyrate	45·4
Riboflavin tetracaprate	3·4
Riboflavin tetrapalmitate	1·2

Reaction mixture: 0·5 ml. of 4 mmole/l. substrate suspended in 1 per cent polyvinyl alcohol, 0·5 ml. of the enzyme (20 mgm. protein/ml.) and 1·0 ml. of McIlvaine buffer, *p*H 7·3. Incubation: 37° C., 2 hr.

Fatty acids liberated from these esters by the enzyme were checked. For example, in the case of tetrabutyrate, liberated fatty acid in the incubated reaction mixture was extracted with ethyl ether, concentrated, and then analysed by paper chromatography followed by coloration with bromocresol purple[5]. A single spot corresponding to butyric acid was detected.

From these results it was clear that these fatty acid esters of riboflavin could be hydrolysed to riboflavin and the corresponding fatty acid by the enzyme action of pancreatic lipase. Considering also the fact that these esters are effective as riboflavin by the test of animal growth[6], it is considered that these esters may be converted to riboflavin and then act as a vitamin.

It may be noticed that pancreatic lipase can also attack these newly synthesized esters besides substrates hitherto known.

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Neutral 17-Ketosteroids in Porcine Urine

In searching for an experimental animal which, in regard to its steroid metabolism, would be suitable for an investigation into the mechanism of action of certain synthetic steroids, particularly 19-norsteroids, we decided to use the domestic pig. A few preliminary experiments were made, during which we determined, among other things, the concentration of Zimmermann chromogens in the urine of male and female pigs and in the urine of castrated animals, while the influence of chorionic gonadotrophin and adrenocorticotrophic hormone on the excretion of Zimmermann chromogens in adult male pigs was also determined. After these preliminary experiments we started to isolate and identify the substances which produce the Zimmermann reaction.

For this purpose, 5,760 ml. urine from an adult male animal was extracted by the method of Dingemanse *et al.*[1]. Two extracts were obtained:

(*a*) An extract obtained by heating the unacidified urine to about 80° C., during 4 hr., while simultaneously extracting with benzene.

(*b*) An extract obtained by adjusting the urinary residue resulting from treatment of urine as in (*a*) to *p*H 1, after which it was heated to about 80° C., for 4 hr., while simultaneously extracting with benzene.

The former extract, which contained about 660 mgm. 'dehydroepiandrosterone equivalents', was purified by the Girard method, by chromatography over aluminium oxide and over cellulose powder. In this way we obtained four different Zimmermann positive fractions, two of which have so far been completely purified and identified. The first fraction to be isolated was 3-chloro-androst-5-ene-17-one, which was identified as follows:

(1) By paper chromatography (system propylene glycol: methanol/heptane).

(2) By determination of the melting-point (148–152° C.) and the mixed melting-point with authentic 3-chloro-androst-5-ene-17-one (with the aid of the Eimer and Amend apparatus, uncorrected).

(3) By establishing that no acetate is obtained at acetylation.

(4) By infra-red analysis: the infra-red spectrum was completely identical with that of authentic 3-chloro-androst-5-ene-17-one.

(5) By elementary analysis, which yielded the following results: C = 74·68 per cent, calculated for $C_{19}H_{27}OCl$: 74·39 per cent; H = 8·69, calculated for $C_{19}H_{27}OCl$: 8·81; Cl = 11·35, calculated for $C_{19}H_{27}OCl$: 11·58. The extract of 5,760 ml. urine was estimated to contain more than 300 mgm. 3-chloroandrost-5-ene-17-one.

The second 17-ketosteroid found to be present in urine of male pigs is dehydroepiandrosterone, which was identified as follows:

(1) By paper chromatography (system propylene glycol: methanol/heptane).

(2) By determination of the melting-point (138–142° C.) and the mixed melting-point with authentic dehydroepiandrosterone.

(3) By acetylation, which yielded an acetate which was, as judged by its infra-red spectrogram, melting-point and mixed melting-point, identical with authentic dehydroepiandrosterone acetate.

(4) By infra-red spectrometry: the infra-red spectrum was completely identical with that of authentic dehydroepiandrosterone.

(5) The substance produces the reaction with phosphomolybdic acid which, carried out under certain conditions, is specific of unsaturated steroids.

A total quantity of 77·3 mgm. pure dehydroepiandrosterone was obtained from 5,760 ml. urine.

The urinary extract obtained at *p*H 7 in addition contains 1–2 other 17-ketosteroids oxygenated on C_{11}.

The extract obtained at *p*H 1 has so far been examined only with the aid of paper chromatography. It contains several Zimmermann chromogens. The chromogen present in the highest concentration has an R_P value with paper chromatography = R_F dehydroepiandrosterone, or epiandrosterone, respectively (system propylene glycol: methanol/heptane).

We are indebted to N.V. Organon, Oss, Netherlands, from whom we received authentic dehydroepiandrosterone and authentic dehydroepiandrosterone acetate.

We received a quantity of authentic 3-chloroandrost-5-ene-17-one from the Steroid Reference Collection, London, through the courtesy of Prof. W. Klyne. The infra-red spectra were registered under the supervision of Dr. K. v. Lier, of the National Institute of Public Health, Utrecht. We thank Mr. P. Hubers, of the Organic-Chemical Laboratory of the Municipal University of Amsterdam, for carrying out analytical work.

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Oxytocinase Activity of Plasma of Pregnant Women

It has been shown[1] that plasma or serum from pregnant women has the power of inactivating oxytocin when incubated with the hormone. This power is thought to be dependent on an enzyme, called oxytocinase. The enzyme inactivates both natural and synthetic oxytocin[2–5]. The rate at which plasma inactivates oxytocin increases with the age of pregnancy[1,4,6–9]. Maximum values are said to be reached around the 36th week of pregnancy, after which they remain at the same level until delivery. After parturition the oxytocinase activity decreases progressively, becoming very low in about one month.

Most of the work on oxytocinase has been performed with biological assays with oxytocin as substrate.

According to Tuppy *et al.*[10], oxytocinase is an aminopeptidase with the power of splitting the peptide-bond between tyrosine and cystine in the oxytocin molecule. With regard to this mode of action, Tuppy *et al.* synthesized a substrate, L-cystine-di-β-naphthylamide, with partly the same molecular configuration as oxytocin and worked out a chemical method for the assay of the oxytocinase activity with this synthetic substrate[11].

The results reported by Tuppy *et al.* with their chemical method are different from those obtained by other authors using biological methods. It must, however, be pointed out that even the values reported by different authors using biological assays are indeed different; the activity at the end of pregnancy is reported to be either 1,000 times or 10 times higher in comparison with the activity at the beginning of pregnancy[9,12]. According to Tuppy *et al.* the oxytocinase activity at the end is about 28 times higher in comparison with the values in the non-pregnant state. These authors could not confirm the results, outlined in the introduction, that maximum values are reached around the 36th week of pregnancy with no increase thereafter. Their values show a steady increase up to the time of delivery. These differences in the values reported probably depend on experimental error.

The present preliminary report deals with the results obtained in assays of oxytocinase activity in pregnant women with both the chemical and a biological method in parallel.

A total of about sixty healthy pregnant women were examined, plasma being the enzyme source and syntocinon and L-cystine-di-β-naphthylamide respectively the substrates. The enzyme reaction occurred under carefully standardized conditions, and the determination of the remaining oxytocic activity was made on rat uterus preparations using a four-point

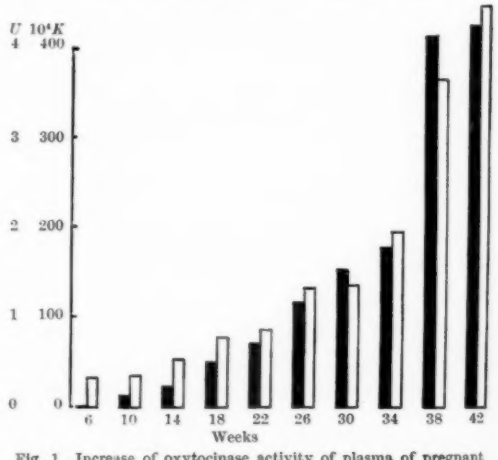

Fig. 1 Increase of oxytocinase activity of plasma of pregnant women in relation to the length of pregnancy. Mean value for each lunar month. Filled bars represent activity determined with biological method and unfilled bars with chemical method

assay with statistical treatment according to Gaddum[13], and Borth *et al.*[14]. Enzyme activity was expressed as $10^4 \times K$; K = the velocity constant according to the formula $K_1 = \frac{1}{t} \ln \frac{a}{a-x}$. In using Tuppy's chemical method I have expressed the enzyme activity as units (U) of Tuppy, 1 mgm. liberated β-naphthylamine per 100 ml. plasma per hr. adopted as 1 U. All estimations were performed in duplicate.

Fig. 1 shows the increase of plasma oxytocinase activity with the length of pregnancy, the activity in each case being determined with biological and chemical method in parallel.

As may be seen, the values obtained with the two methods show on the average the same increase of oxytocinase activity in relation to the length of pregnancy. The results make it probable that the two methods determine identical enzyme activities; this has been questioned earlier. The activity also shows a steady increase up to the time of delivery, which is in agreement with the results of Tuppy *et al.*[11].

Work is now in progress in order to secure these preliminary results and investigate the enzyme activity in abnormal conditions of pregnancy. Attempts to concentrate and purify the enzyme are also in progress.

I have not had the opportunity of investigating closer the kinetics of the enzyme reaction. It may be that the reaction is of zero order; but the values of K_0 according to the formula $K_0 = \frac{x}{t}$ differ little from the values of K_1 and the increase of enzyme activity, expressed as K_0, is the same as that expressed as K_1. The kinetics of the reaction are being investigated.

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Inhibition of Chemi-luminescence of Luminol by means of Oximes

IT is known that oximes with widely different chemical structures are effective reactivators of cholinesterase inhibited by organo-phosphorous compounds (esters of phosphoric or thiophoric acid, insecticides, nerve gases)[1]. The mechanism of this reaction is considered to be due to the chemical interaction of oximes with the free or bound ester of phosphoric acid, which is hydrolysed by the oxime[2], and is not available for the inhibition of the cholinesterase. It is known that organo-phosphorous compounds catalyse the luminol reaction causing an intensive chemi-luminescence of luminol (3-aminophthalylhydrazide) in alkaline medium in the presence of sodium perborate as oxygen donor[3].

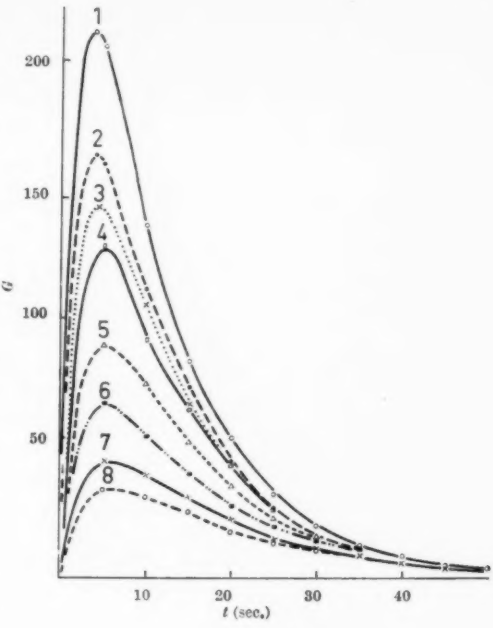

Fig. 1

In view of this evidence it was felt that the oximes will inhibit the luminol reaction catalysed by organo-phosphorous compounds or other suitable catalyst, that is, hæmoproteins or other complexes with iron. This type of inhibition would proceed with a mechanism analogous to the reactivation of cholinesterase as mentioned here.

Quantitative investigations of the chemi-luminescence of luminol have proved that a large number of oximes inhibits the luminol reaction catalysed by organo-phosphorous compounds or complexes of iron. The inhibition caused a significant decrease of the chemi-luminescence. The results of a series of experiments are presented (Fig. 1). G represents the relative intensity of chemi-luminescence in relation to the reaction time t. Curve 1 records the results obtained

Table 1

Inhibitor \ Hæmoglobin (gm. per cent)	2×10^{-5}	4×10^{-5}	1×10^{-4}
	$i_{1/2}$ M		
2-Hydroxyiminopropane	$5{\cdot}90 \times 10^{-2}$		$6{\cdot}10 \times 10^{-2}$
4-Hydroxyiminomethyl-N-methylpyridinium iodide	$1{\cdot}34 \times 10^{-2}$		$1{\cdot}54 \times 10^{-2}$
2-Hydroxyiminobutanone-(3)	$1{\cdot}11 \times 10^{-2}$	$1{\cdot}25 \times 10^{-2}$	$1{\cdot}40 \times 10^{-2}$
2-Hydroxyiminomethyl-N-methylpyridinium iodide	$2{\cdot}25 \times 10^{-3}$	$2{\cdot}50 \times 10^{-3}$	$3{\cdot}25 \times 10^{-3}$
N,N'-Tetramethylene-*bis*(4-hydroxyiminomethylpyridium bromide)	$6{\cdot}90 \times 10^{-4}$	$7{\cdot}10 \times 10^{-4}$	$7{\cdot}80 \times 10^{-4}$

XUM

without oxime, while curves 2–8 illustrate the effect of increasing concentrations of pyridine-4-aldoxime methiodide. In all these experiments the luminol reaction was catalysed by hæmoglobin. The results clearly demonstrate that the maximal intensities of chemi-luminescence as well as the total amount of light (area under each curve) were reduced when increasing amounts of oxime were used.

Essentially the same results were obtained when other oximes (inhibitors) and other catalysts of luminol reaction were applied. Table 1 shows molar concentrations of various oximes which decrease the chemi-luminescence to a value of 50 per cent at various concentrations of hæmoglobin. It is evident that oximes inhibit the luminol reaction effectively.

These findings present not only an additional proof for the mechanism of reactivation of cholinesterase with oximes, but also the method described in this communication provides a means for a simple evaluation of oximes as reactivators of cholinesterase.

Further details of these and other experiments on related subjects will be published elsewhere.

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Inactivation and Reactivation of Chymotrypsin

In previous work on the immediate effects of acylation of trypsin it was shown[1] that the enzyme can be obtained in a fully inactive form by acylation with various anhydrides at *p*H 8–9, and that partial instantaneous reactivation can be effected by treatment with alkaline hydroxylamine[2].

In an extension of this work it was found that chymotrypsin behaves similarly to trypsin on acylation at higher *p*H values, complete inactivation occurring at *p*H 8. Even though a reactivation was observed on incubation, it was almost negligible in the course of 2 hr. The acylations were carried out using an automatic titration unit (Polarad Electronics Corp.), at various constant *p*H values. In Table 1 are recorded the percentages of inactivation and reactivation in terms of proteolytic activity obtained on acetylation at 0–5° C. at different *p*H values, whereby 60 equiv. of acetic anhydride were used per mole of enzyme. The activity was assayed at *p*H 7·8, using the usual hæmoglobin substrate. Any inactivation rapidly reversed at that *p*H [3,4] could accordingly not have been observed.

Table 1. Inactivation of Chymotrypsin at Different *p*H Values

Time (min.)	Activity (per cent) 5	6	7	8
0	100	100	100	100
8	65	56	31	0
20	64	58	35	0
120	67	60	38	0

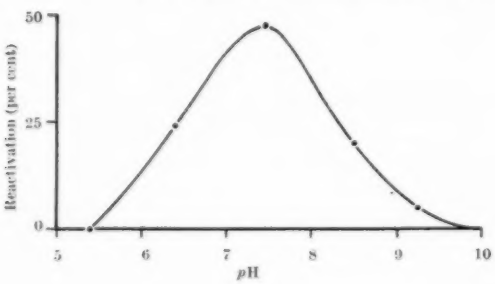

Fig. 1

Butyric and propionic anhydrides gave similar results. As observed in the case of trypsin, citraconic anhydride gave rise to an inactivation amounting to only about 50 per cent.

Even though the spontaneous reactivation of acetylchymotrypsin is much slower than in the case of acetyltrypsin, an instantaneous reactivation was obtained on treatment with hydroxylamine. In Fig. 1 is shown the extent of reactivation recorded at various *p*H values on treatment of the inactivated chymotrypsin for a period of 8 min. with 4 equiv. of hydroxylamine per equivalent of anhydride added. It can be seen that maximum reactivation takes place in the region of *p*H 7·4–7·8. The reactivation bears a striking resemblance to the reaction of inactive acetyltrypsin with hydroxylamine[2] except that the *p*H optimum of reactivation is shifted to lower *p*H values by about one *p*H unit.

The inactive monoacetyl chymotrypsin obtained by other investigators[3,4] was reactive with hydroxylamine in the region of *p*H 5–6. In reaction with nitrophenylacetate there is also a great similarity between trypsin and chymotrypsin and the monoacetyl enzymes obtained were more stable at *p*H 5–6, easily undergoing hydrolysis at *p*H 7–8. However, in the case of the inactive acetyl derivatives obtained at *p*H 8, it was found that they were more stable at *p*H 7–8 than at *p*H 5–6. The hypothesis[2] that there are two susceptible sites in the active centre of trypsin, acetylated at *p*H 5 and *p*H 8 respectively, appears to be supported in the case of chymotrypsin. The small deviations in the *p*H optima of reactivation with hydroxylamine could be attributed to secondary differences between the two enzymes.

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Activity of Glutamic Acid Decarboxylase in Insect Nerve Tissue

As compared with the nerve tissue of mammals, that of insects is known to be particularly rich in acetylcholinesterase[1] and in choline-acetylase[2]. On the other hand, nothing has been reported so far about the occurrence in insects of another enzyme which, in mammals, is characteristic of nerve tissue, namely, glutamic acid decarboxylase. Its reaction

product, γ-aminobutyric acid, has been recently found in the free state in bee nerve tissue, in amounts of the same order, on the basis of fresh weight, as in mammalian nerve tissue (Carta, S., and Frontali, N., unpublished work).

Homogenates of worker honeybee brains (including the optic lobes) have been used for determinations of glutamic acid decarboxylase by the manometric technique[2], and compared with mouse brain homogenates. The average values thus obtained were 690 μl. carbon dioxide/hr./gm. of fresh tissue for the mouse and 1,250 for the bee.

Further work on the characteristics of the glutamic acid decarboxylase of bee nerve tissue was made difficult by the large amounts of material necessary for each determination, even when Warburg microvessels were used (at least 0·1 gm. tissue, which is approximately 80 brains). For this reason a different method of assay of glutamic acid decarboxylase was worked out and found suitable for activity determinations on as little as 2–4 mgm. of tissue. It was based on the use of uniformly labelled glutamic acid-^{14}C as substrate, and on the measurement of the radioactivity of the γ-aminobutyric acid produced.

Preliminary experiments showed (1) that labelled glutamic acid, incubated with a bee brain homogenate under the same conditions as the Warburg determination and subjected to two-dimensional paper chromatography for amino-acids, gave rise to one new radioactive spot, having the R_F values of γ-aminobutyric acid; (2) that this spot, eluted and co-chromatographed with authentic inert γ-aminobutyric acid, could be superimposed perfectly on the resulting ninhydrin spot.

The general procedure was as follows, each determination being run in duplicate. Small test-tubes (30 mm. × 6 mm.) were pre-cooled in ground ice with 20 μl. of the reaction mixture, which was prepared in such a way as to give a final concentration of 0·0005 *M* pyridoxal phosphate and 0·025 *M* L-glutamic acid; each test-tube contained 0·2 μc. of labelled L-glutamic acid (from the Radiochemical Centre, Amersham, England). Next, 20 μl. of bee brain homogenate 1 : 5 in 0·1 *M* phosphate buffer *p*H 6·4 were added. The test-tubes were closed with rubber stoppers, and by means of two injection needles (inlet and outlet) through the stopper, pure nitrogen was passed through them for 2 min.; the needles were quickly removed at the end of the gassing period. At zero time the test-tubes were immersed in a water-bath at 38°, except for one which was immediately deproteinized to serve as a control. After incubation for 20 min., the test-tubes were chilled on ice, opened and immediately deproteinized with 100 μl. of 99 per cent ethanol. After centrifugation, measured aliquots of the supernatant fluids were subjected to one-dimensional ascending paper chromatography in phenol/water/concentrated ammonia (77·5 : 21·5 : 1). The radioactivity was measured by the quantitative paper radiochromatographic technique described by Beloff-Chain *et al.*[4]. The results obtained by this method were found to agree with those obtained by the manometric technique.

The above method was then applied to the study of some properties of the glutamic acid decarboxylase of bee brain. Enzyme activity, as a function of coenzyme concentration and of time, was examined. The results of these experiments showed that (1), the optimal concentration of pyridoxal phosphate is between 4 × 10^{-4} and 10^{-3} *M*, (2) the time/activity curve is almost linear during the first 20 min.

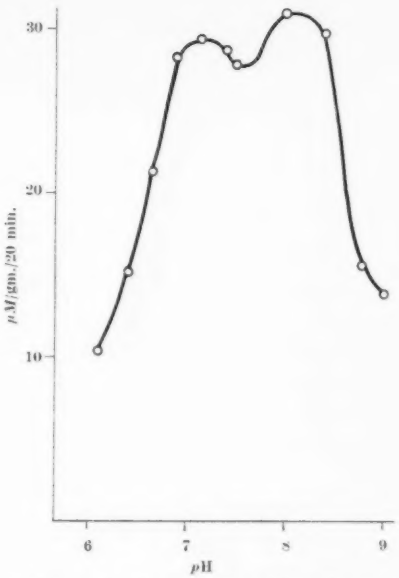

Fig. 1. Production of γ-aminobutyric acid from glutamic acid as a function of *p*H

Enzyme activity as a function of *p*H was then investigated. A homogenate of bee brain in 0·1 *M* potassium chloride was incubated with the usual reaction mixture and with a phosphate-borate buffer[5] ranging from *p*H 6 to 9. The amounts of γ-aminobutyric acid formed were determined in the usual way and plotted against the *p*H. The resulting curve showed two peaks of maximum γ-aminobutyric acid production at *p*H values around 7·2 and 8·0 respectively (Fig. 1). The second peak could correspond to an alternative route of γ-aminobutyric acid production, in which glutamic acid decarboxylase is not involved. If this can be shown to be true, the method described in this communication should be used as a measure of glutamic acid decarboxylase activity only between certain limits of *p*H.

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Enzymic Conversion of Tetrahydrofolic Acid to Folinic Acid by Plant Protein

Enzymic synthesis of folinic acid (CF) has been investigated in a variety of cells of both bacterial and animal origin[1–7].

The purpose of this communication is to report the isolation and partial characterization of a crude protein fraction from a plant source demonstrating 'transformylase' activity in converting tetrahydrofolic acid to folinic acid.

Folic acid was obtained from the Nutritional Biochemical Corporation, Cleveland, Ohio. Formylfolic acid (FF) was prepared by the method of Shive *et al.*[8]. Identity was verified by the method of Weislogel *et al.*[9]. Tetrahydrofolic acid (THF) was prepared by an adaptation of the method of O'Dell *et al.*[10]. Folinic acid-SF was supplied through the courtesy of Eli Lily Co., Indianapolis, Indiana. Mustard leaves were obtained locally.

Turnip or mustard leaves (2·5 kgm.) were homogenized in a Waring blendor in a v/v solution of acetone chilled with dry ice and phosphate buffer (0·05 *M*) at *p*H 7·2. The homogenate was collected by filtration; acetone was removed by vacuum distillation, and the residue was extracted with 500 ml. phosphate buffer at *p*H 7·2.

The volume was reduced to 250 ml. by pervaporation and the debris which accumulated was removed by centrifugation. Protein was precipitated by adding ammonium sulphate (42 gm./100 ml. volume).

Further purification of the fraction was accomplished by suspending the protein precipitate in a minimum of buffer, clarifying the suspension by centrifugation, and by discarding the small amount of insoluble precipitate. The centrifugate was reprecipitated with ammonium sulphate at the same saturation-level as used in the precipitation of the crude fraction. The precipitate was collected by centrifugation, dissolved in buffer, and dialysed against potassium phosphate buffer until no precipitate occurred on the addition of 0·1 *M* barium chloride to 5 ml. of the buffer bath. The protein was dissolved in phosphate buffer to give 63·20 μgm. in 0·2 ml. of solution. Protein determination was by the method of Lowrey[4].

The biosynthesis media contained the following substances: tetrahydrofolic acid, 2 μgm.; glucose, 8 μ*M*; ascorbic acid, 4 μ*M*; sodium formate, 20 μ*M*; $MgSO_4.7H_2O$, 1 μ*M*; diphosphopyridine nucleotide, 0·05 μ*M*; adenosine triphosphate, 0·05 μ*M*; protein fraction, 0·1 mgm.; buffer (potassium phosphate *p*H 7·0), 1·0 ml. in a total volume of 4·0 ml.

After 4 hr. incubation at 30° C., the biosynthesis samples were clarified by centrifugation, the *p*H adjusted to 7, and assayed for folinic acid activity with *P. cerevisiae* (*ATCC* 8081) in a manner described previously[2]. Samples containing no protein, as well as samples containing only protein, gave no stimulation of growth of *P. cerevisiae*, when assayed under identical conditions as the biosynthesis samples.

Substrate specificity for this enzyme was estimated by using formyl-THF, THF, and folic acid. THF gave the best conversion to folinic acid, although, in each case, the yield of folinic acid was low, due, perhaps, in part to substrate instability, unacceptability of D-forms of the stereoisomers, and the impure condition of the enzyme. In determinations of the effects of *p*H, and temperature, 2 μgm. of THF per 5 ml. of biosynthesis medium were employed, and the amount of protein was reduced from 0·1 mgm. to 0·03 mgm.

Because of the observed influence of both temperature and *p*H (Table 1), as well as the absence of activity of folinic acid, when no protein was present, it can be said that the conversion of THF to folinic acid by this plant protein is enzymic.

These results show the normally observed inactivation of enzyme activity at extremes of, as well as the optimum, activity within the mid-range of *p*H and temperature, which compares favourably with observations of effects of *p*H and temperature on similar enzymic conversions of folic acid derivatives to folinic acid in both bacteria and animals.

Table 1. Effect of *p*H and Temperature on the Enzymic Conversion of Tetrahydrofolic Acid to Folinic Acid

*p*H	Conversion (per cent)*	Temperature (° C.)	Conversion (per cent)†
4·0	0	10	0
6·0	12	25	8
8·0	12	37	16
10·0	2	90	0

* Percentage conversion of tetrahydrofolic acid to folinic acid per mgm. of crude protein at 30° C. Incubation time, 4 hr. Assays for folinic acid activity were for 18 hr. at 37° C.
† *p*H 7.

Further purification of the active fraction is necessary in order to make a thorough study of the properties of this enzyme.

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Regulation of Pyrogallol Oxidations by Alcohols and Sugars

Previously, it has been shown that the catalytic activity of Fe(III) or peroxidase can be enhanced by a variety of colloidal substances including deoxyribonucleic acid[1], celluloses[2], and starch[3]. Other investigations[4–6] have demonstrated the macromolecular activation or modification of a number of oxidative or hydrolytic systems. Such observations direct attention to the role of environmental factors other than those usually encountered *in vitro* in biochemical reactions.

Although investigations of this type are not common, they possess potentially great significance as demonstrations of the regulatory effects to be expected when reactions take place within the colloidal system of the cell. Such investigations also stimulate interest in still other kinds of model environments which might possess biological counterparts. We may consider, for example, the properties of the solvent medium wherein reactions occur, such as dielectric constant and viscosity. Using alcohols and polyhydric substances such as glycerol and sugars as solvent-modifying solutes, it has been found that the autoxidation and enzymic oxidation of pyrogallol can indeed be regulated by solvent properties.

In these experiments, the oxidation of 0·05 *M* pyrogallol at 25° C. in 0·01 *M* phosphate buffer, *p*H 5·6, was followed photometrically at 425 mμ. In the higher alcohol concentrations, buffer salts were omitted.

It is known that the autoxidation-rate declines in pure alcohols with increasing length of carbon chain[7],

Table 1. Effects of Alcohols on Autoxidation of Pyrogallol

(*a*) Simple alcohols

Concentration (*M*)	Methanol	Ethanol	*n*-Propanol	Glycerol
0*	100	100	100	100
0·01	—	198	350	226
0·1	175	200	330	253
1·0	152	233	350	253
5·0	101	110	153	150
10·0	—	64	14	33

(*b*) Sugars

	Sucrose	Glucose
0†	100	100
0·01	88	88
0·1	119	94
0·5	123	—
1·0	105	100
2·0	32	65
5·0	—	16

*, In water, the control absorbance at 425 mμ after 24 hr. is 0·152; †, in water, the control absorbance at 425 mμ after 50 hr. is 0·450.

but the enhancement of oxidation by dilute aqueous alcohols was unexpected (Table 1*a*). Several alcohols at 0·01–5 *M* cause pronounced acceleration and at concentrations in excess of 5 *M* pronounced inhibition.

Glycerol acts like the monohydric alcohols; however, other polyhydric substances, sucrose and glucose (Table 1*b*), affect only the inhibition of autoxidation when supplied at concentrations greater than 1 *M*. On the basis of their molar concentrations, the sugars are more inhibitory than the simple alcohols. At 5 *M*, the simpler alcohols are inactive or stimulatory whereas both sucrose and glucose inhibit autoxidation at the 2 *M* concentration.

The theoretical and mechanistic bases for these effects will be discussed elsewhere, and it will suffice here to mention several possibilities in outline: (1) Enhancement by dilute ethanol and *n*-propanol may involve participation in radical chain transfer reactions. (2) Inhibition by more concentrated alcohols may involve reduced ionization of pyrogallol in media of much lower dielectric constant than water. Inhibition by ethanol and *n*-propanol specifically may also involve their radical chain-stopping (anti-oxidant) activities. (3) Enhancement by methanol and glycerol does not now lend itself to explanation. (4) Inhibition by the sugars and glycerol may involve the retarding effect of viscous media. The non-inhibitory concentrations give solutions of 5 centipoise or less, whereas the viscosities of inhibitory solutions range upwards from 50 centipoise.

Some of the effects described are not restricted to homogeneous autoxidation systems, but may be extended to enzymic peroxidations as well. Using the peroxidase system described in previous work[3], we have found that methanol at concentrations of 5 *M* or less accelerates pyrogallol oxidations appreciably (Table 2).

Table 2. Effects of Methanol on Enzymic Peroxidation of Pyrogallol

Methanol *M*	Yield of purpurogallin (mμmoles/l.) at 0·5	1·0	1·5	2·0	2·5	3·0 min.
0	11·3	23·7	33·8	38·8	44·0	48·1
2·5	8·1	19·4	34·4	47·5	62·5	66·0
3·75	5·6	14·4	27·5	39·4	53·8	62·5
5·0	4·1	9·1	17·2	28·1	40·6	53·1
7·5	2·8	5·6	9·4	13·8	18·8	25·0
10·0	1·9	4·7	7·8	11·0	14·7	18·5
12·5	1·0	1·3	2·0	2·8	3·5	4·4

At concentrations of 0·5 *M*, sugar solutions inhibited enzymic oxidation by 15–20 per cent, but at 5 *M* the inhibition was increased to about 50 per cent. Hence, methanol and the sugars exert similar effects on the slow reaction between pyrogallol and oxygen and the fast catalytic reaction between pyrogallol and hydrogen peroxide. The biological significance of these results lies mainly in the principle illustrated, namely, that modifications in aqueous reaction media brought about by the presence of various simple hydroxylic substances can influence the rates of oxidative reactions.

Such effects could give rise to differences in rate among cells or parts of cells without requiring corresponding differences in catalyst or reactant concentrations.

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PHYSIOLOGY

Importance of Magnesium in the Control of Plasma Calcium in the Calf

It has been known for many years[1] that when calves are fed for an abnormally long time on milk only they usually, sooner or later, become hypomagnesæmic. In my experiments, four out of sixteen calves fed to about five months of age or more on milk without supplementary vitamin D showed neither hypomagnesæmia nor hypocalcæmia while the remainder became hypomagnesæmic. Of the latter, all but one showed a fall in plasma calcium more or less closely correlated with the fall in plasma magnesium. An example is shown in Fig. 1 and other examples have been reported previously[2]. My work has shown fairly clearly that the hypomagnesæmia is due to magnesium deficiency resulting from a decrease in the ability of the calves to absorb dietary magnesium as they get older[3], but the reason for the hypocalcæmia is less clear.

I have found that the efficiency of calcium utilization as well as that of magnesium decreases as the calves get older and that addition of a large amount of vitamin D (70,000 I.U./day) to the diet both improves calcium utilization and rapidly restores plasma calcium to normal[3]. Smaller amounts of vitamin D (300–7,000 I.U./day) have also been found to be beneficial, but less so than the larger amount[3].

Table 1

Age (weeks)	Diet	Magnesium intake (gm./day)	Calcium intake (gm./day)	Calcium net absorption (per cent of intake ± S.E.)	Calcium excretion in urine (per cent of intake ± S.E.)	Plasma magnesium (mgm./100 ml.) mean and range	Plasma calcium (mgm./100 ml.) mean and range
16½–18½	Milk	0·69	7·1	61 ± 6	0·35 ± 0·09	1·35 (0·91–2·08)	8·5 (7·4–10·6)
19½–21½	Milk + $MgCl_2$	4·0	7·1	42 ± 11	1·44 ± 0·74	2·34 (1·94–2·54)	10·8 (9·4–12·7)

Mean results from 8-day balance experiments with 4 calves kept out of direct sunlight and previously fed only on milk (4·4–6·15 l./day with supplementary iron, copper and manganese) throughout their lives. Immediately after the first balance period, the magnesium chloride supplement was added to the diet and subcutaneous magnesium sulphate injections (1 gm. magnesium/day) were given for 7 days. The second balance period was started 4–6 days after the injections ceased. General procedure and analysis was carried out as described previously (ref. 2).

Now milk, particularly winter milk which is what the calves here received, contains little vitamin D (about 10 I.U./l.); but, although it is clear that the vitamin D level must be low for the hypocalcæmia to occur, there are a number of facts which suggest that vitamin D-deficiency is not its sole cause. First, calves on vitamin D-deficient but otherwise normal diets do not readily show hypocalcæmia, even though they may suffer bone changes and show clinical signs of rickets[4]. Secondly, deficiency of vitamin D cannot explain the correlation between falls in plasma magnesium and plasma calcium; plasma magnesium and magnesium absorption are not appreciably influenced by vitamin D (ref. 2). Finally, I have now found that a restoration of plasma calcium to nearly normal levels occurred in four milk-fed hypomagnesæmic and hypocalcæmic calves when the plasma magnesium was restored by giving oral and parenteral supplements of magnesium even though no supplementary vitamin D was given. An example of the effect is shown in Fig. 1. With different animals an increased amount of magnesium in the diet has been variously reported as improving[5], inhibiting[6] and having no marked effect on[7] net calcium absorption. To examine the possibility of an improvement in calcium utilization accompanying magnesium supplementation in the above calves, calcium balances were carried out just before daily magnesium supplementation was started and again about ten days after it was started. Mean results are shown in Table 1.

Although the results do not prove that magnesium supplementation rather than a simple age effect caused the decrease in net calcium absorption, it is

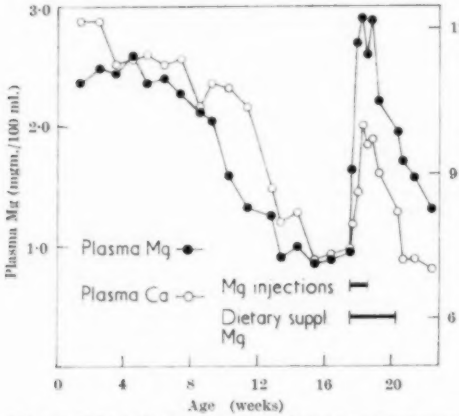

Fig. 1. Calf fed only on milk (4·4–6·15 l./day with supplementary iron, copper and manganese) and kept out of direct sunlight. Dietary supplement of 28 gm. $MgCl_2.6H_2O$/day and daily subcutaneous injections of 10 gm. $MgSO_4.7H_2O$ given as shown. During injection period blood samples were taken 24 hr. after an injection

nevertheless true that an increase in plasma calcium occurred in all these calves in the face of decreased net absorption of dietary calcium and, as a mean effect, increased urine excretion of calcium (in some calves urine calcium increased markedly, in others it remained unchanged). It seems very probable, therefore, that the homeostatic mechanism for calcium, presumably that controlling the bone/extracellular fluid equilibrium, is sensitive, under certain conditions, to the magnesium status of the calf as reflected by the level of plasma magnesium. I have no evidence as to whether magnesium exerts its effect directly on the bone/extracellular fluid equilibrium or otherwise, but bearing in mind that, in the young calf, the plasma magnesium-level reflects the magnesium content of the bone[8], it can be seen that the idea of a direct effect is in accordance with the *in vitro* findings of W. F. Neuman and his co-workers[9] that the presence of certain foreign ions, including magnesium, increases the effective solubility of bone hydroxy-apatite crystals.

Analogous considerations might account for the frequent occurrence of hypocalcæmia in adult bovines developing clinical hypomagnesæmia.

Although some tendency for low plasma calcium to be associated with low plasma magnesium has occasionally been observed by other workers with milk-fed calves (particularly by Parr[10], who often found hypocalcæmia in hypomagnesæmic calves even though they received about 430 I.U. vitamin D/day and who also found that the hypocalcæmia was alleviated by supplementary magnesium), the close correlation which I observed has not been reported previously. Its absence is often explicable by differences in vitamin D intake and might also sometimes be explained by differences in the amounts and types of foreign solid matter eaten by the calves in addition to their milk. Calves kept on fluid diets for longer than a few weeks develop a very strong desire for solid material to chew and will often eat almost anything. The calves used here were kept muzzled and were bedded on wood shavings, but they still usually succeeded in eating a small amount of these shavings.

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Synaptic Transmission in Neurosecretory Cells

SINCE Scharrer's discovery[1], many types of neurosecretory systems have been investigated histologically in almost all kinds of animals. However, there is no publication dealing with them in terms of electrical activities except the brief note of Potter and Loewenstein[2], in which the presence of synapses between neurosecretory cells and other nerve cells was only indirectly indicated.

The caudal neurosecretory system of the eel, *Anguilla japonica*, was found by Enami[3]. In this system the cells are fairly large and clearly seen in fresh material under a dissecting microscope. This fact enabled us to use the micro-electrode technique, and the results obtained with this method clearly showed the synaptic transmission at the cell-bodies as well as other characteristics of the nervous function.

After the caudal spinal column of approximately 20 vertebræ was isolated from the eel, the spinal cord was exposed at several vertebræ including the tail end. This preparation was fixed with 'Vaseline' on a paraffin block in a leucite chamber, and the exposed spinal cord was washed sufficiently with the teleost Ringer. Intracellular records from the cell-bodies of these neurosecretory cells showed resting membrane potentials of 50–60 mV. inside negative and overshoots of 10 mV. or more in action potentials. The duration of the action potential was 8–10 msec. at 14–17° C. and the threshold membrane depolarization was around 12–13 mV. A long lasting outward current pulse resulted in repetitive discharge of spikes, at the end of which an under-shoot of the membrane potential was clearly seen (Fig. 1). These characteristics are very similar to those of the spinal ganglion cells of the frog[4].

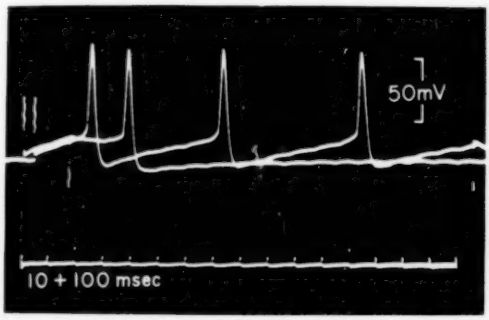

Fig. 1. Spike potentials of a cell body elicited by outward current pulses. The responses to brief and long pulses are superimposed

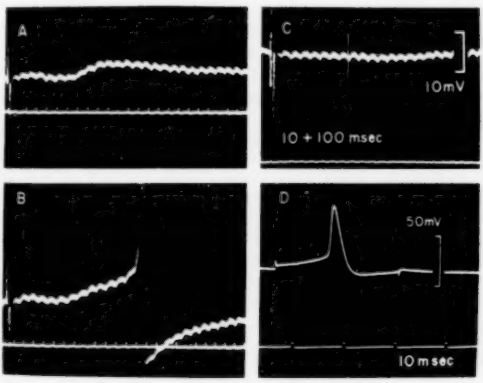

Fig. 2. An experiment showing synaptic transmission. *A*, synaptic potential evoked by a rather weak stimulation of a spinal cord at an anterior part; *B*, synaptic potential followed by a spike in the case of a stronger stimulation; *C*, after treatment of the spinal cord with potassium chloride in a region between the stimulating and recording positions. The stimulus was much stronger than in *B*; *D*, after *C*, the cell was directly stimulated by an outward current

When the spinal cord was stimulated at an anterior segment, a slow potential change was recorded from the neurosecretory cell-body after some delay (Fig. 2*A*). If the stimulus was strong enough, a spike potential was initiated at the summit of the slow potential (Fig. 2*B*). After this spinal cord was treated with potassium chloride in a region between the stimulating and recording positions, no electrical changes were evoked by a stimulus several times as strong as that which could elicit the spike potential (Fig 2*C*). Even then, this cell responded to a stimulus directly applied to the cell-body (Fig. 2*D*). Thus, the slow potential mentioned above may be safely regarded as the synaptic potential, which is also supported by the fact that the temporal summation was clearly observed. The conduction velocity of the fastest fibres in the presynoptic nerve was about 0·6 m./sec. at 15° C., but that of the neuro-secretory cell could not be measured owing to the short length of the axone, though antidromic stimulation of it was possible.

From the results of the present work it may be concluded that the neurosecretory cells have complete nervous characteristics, and that the activities of these cells are controlled by axones from higher nervous centres, though their locations are quite open for further investigations.

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Changes in Blood Serotonin during Exposure to Cold

SEROTONIN is a recently discovered biogenic amine which can be extracted from brain, blood, and other organs, and which is active on a wide variety of systems when injected into the animal[1]. Gathering of knowledge concerning serotonin's physiological importance has been slowed by an inability to observe fluctuations in its concentration in brain and other tissues, in a wide variety of circumstances simulating physiological mobilization[2–4]. A single exception to this is the observation of a release of serotonin into the bowel, controlled by, and in turn modifying, peristaltic activity[5]. Also, more recently, Toh has reported rat brain serotonin to drop following exposure to cold associated with drop in body-temperature of 36–30° C. (ref. 3).

Below I present further data relating serotonin to natural mechanisms, reporting serotonin perturbations in the rat's blood during a short-term exposure to cold, not associated with change in body tem-

perature. 200-gm. female rats were taken from an atmosphere varying from 75° to 80° F. and placed on a metal surface in a cold room maintained at 36–38° F. Animals of exps. 1–3 were injected with 50 mgm./kgm. sodium heparin intraperitoneally to facilitate blood collection, 30–40 min. before the beginning of exposure to cold. At specified intervals, of 15 sec.–20 min. duration, they were decapitated, and bled into cold siliconized test-tubes containing 1 drop of heparin. Adapting a method of Weissbach[6], 0·5 c.c. rat blood was pipetted into 7 c.c. of cold distilled water and lysed, and 2·5 c.c. of a 5 per cent zinc sulphate solution was added with shaking. The tubes were then neutralized with the appropriate quantity of sodium hydroxide, shaken again and centrifuged. The supernatants were treated in one of three ways: they were analysed for 5-hydroxyindole content by direct fluorimetry[6], or extracted and analysed specifically for 5-hydroxytryptamine (serotonin) by combination of the zinc sulphate protein precipitation[8] with the Bogdanski extraction procedure[7], one very close to Weissbach's extraction method, or, they were split, and both direct and extraction analyses carried out on the same sample. The fluorimetric readings of samples and recoveries were performed on a Farrand spectrophotofluorimeter. Samples were found to have emission and absorption spectra identical with processed serotonin.

Table 1. Effect of Cold Exposure on Whole Blood Serotonin

Exp.	No. of samples	Duration cold exposure	Whole-blood γ/c.c. 5-hydroxyindole		P
			Direct method	Extraction method (serotonin)	
1 Rat (heparin)	5	0 min.	0·94 ± 0·24	0·43 ± 0·09	
	4	5–7 ,,	1·77 ± 0·20	1·08 ± 0·22	< 0·01
	4	9–11 ,,	1·63 ± 0·33	1·08 ± 0·17	
	4	20 ,,	1·44 ± 0·50	0·72 ± 0·23	
2 Rat (heparin)	16	0 min.	0·95 ± 0·21	—	
	18	15–75 sec.	1·39 ± 0·24	—	
	8	2 min.	1·40 ± 0·34	—	< 0·001
	8	4 ,,	1·09 ± 0·26	—	
	5	6 ,,	1·01 ± 0·12	—	
	5	11 ,,	0·94 ± 0·28	—	
3 Rat (heparin)	3	0 min.	1·95 ± 0·67	1·77 ± 0·88	—
4 Rat (no heparin)	10	0 min.	—	1·52 ± 0·46	< 0·01
	10	5–9 ,,	—	2·20 ± 0·49	
5 Rabbit	7	0 min.		3·29 ± 0·86	
Human	7	0 ,,	—	0·12 ± 0·06	—
Rat	8	0 ,,	—	1·41 ± 0·29	

The results are described in Table 1. Exp. 1 depicts increases in blood 5-hydroxyindole and serotonin that are approximately equivalent, about 0·7γ serotonin/c.c. whole blood. This elevation begins falling away between the 11th and 20th minute. Exp. 2 examines the same phenomenon by the direct method only. In this experiment the rise and fall of blood 5-hydroxyindole follows a more rapid time course. Exps. 3 and 4, performed a month later, demonstrate an equivalent cold-induced serotonin elevation in animals not injected with heparin. In these experiments, the normal values are seen to be somewhat high in comparison with the values obtained in other experiments for the rat[8,9]. In contrast, as shown in Exp. 5, human and rabbit bloods analysed simultaneously with rat samples gave serotonin titres similar to those obtained by other methods[6,8–11]. No other laboratories report whole-blood analyses for the rat, all blood serotonin values for rats reported to date being derived by calculation from analysis of platelets or platelet-rich plasma[6,8–11]. Thus the somewhat higher normal values we observe remain to be explained: they may reflect a seasonal variation, an inefficiency of methods for gathering platelets in the rat, or, possibly, the existence of a non-platelet source of blood serotonin in this species.

In contrast to Toh's observations[3], it is important to note that the alterations we have observed could not have been provoked by a non-specific lowering of body temperature. Rectal temperatures were not significantly altered in rats exposed to cold for 30 min.

The question of the source of the increase in blood serotonin and the relation of this observation to a local and general increased serotonin metabolism are under investigation at present. We speculate that the perturbations in serotonin concentration observed indicate serotonin participation in cardiovascular and/or metabolic adjustments to cold.

Note added in proof. In recent experiments employing blood drawn by cardiac puncture a cold-induced increase in plasma serotonin was not observed.

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Effect of Sodium Fluoride on Calcium Metabolism of Human Beings

Although bones of human subjects become abnormally dense after several years of industrial exposure to fluorides[1,2], no work on the effect of fluoride on calcium balance of human beings has been carried out. Roholm[1] and others[2] have shown that the severity of skeletal changes is correlated approximately with the extent and duration of exposure to fluoride: moderate exposure resulting in increased density of otherwise grossly normal bone, but prolonged heavy exposure resulting in marked changes, including periosteal bone-formation and calcification of ligaments and tendons. How such complex skeletal effects are brought about is unknown; however, the microscopic appearance of depressed or absent osteoclastic activity in bones of moderately affected human beings[1] suggests that relatively low doses of fluoride may somehow depress resorption of bone.

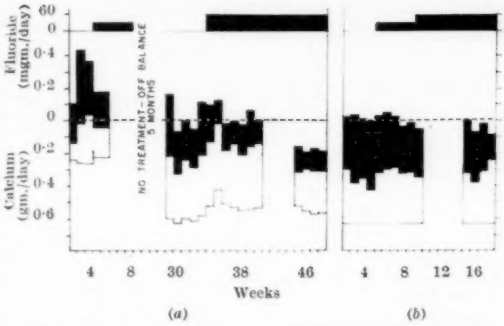

Fig. 1. Calcium balances of a patient with widespread and active Paget's disease (*a*) and of a patient with inactive post-menopausal osteoporosis (*b*). Data are plotted after Albright with intake down and urinary above fæcal calcium

Because of the demonstrated skeletal effect of fluoride in human beings, and because of the relatively benign nature, even of advanced fluorosis[1], it is possible that fluoride could be of use in treatment of diseases where skeletal mass is reduced, the rate of bone resorption accelerated, or both. This work was carried out to determine whether or not metabolic effects, particularly retention of calcium, could be observed during treatment of human beings with sodium fluoride. Six patients with osteoporosis and one with Paget's disease were given carefully measured diets which contained approximately constant amounts of calcium, phosphorus and nitrogen. After a preliminary period of at least one week, stools and urine were collected and balance measurements carried out. Control observations were made, usually for three weeks, after which the patients were given sodium fluoride orally in divided doses (all other treatments remaining unchanged). In early work small doses of fluoride were used and treatment was given for only a few weeks. As it was found that the medication was well tolerated, and that effects observed were seen only after several weeks, the dose was increased to 60 mgm. fluoride a day and duration of treatment extended to 14 or more weeks.

Changes in balance of calcium (the analysis[2] for calcium was unaffected by addition of sodium fluoride) of a subject with widespread and progressive Paget's disease are illustrated by Fig. 1*a*. The decreased rate of excretion of calcium in urine and the calcium retention were most marked late in the investigation, and did not appear to have become maximal during the period of observation. Fig. 1*b* shows similar but less-striking results from study of a post-menopausal patient with minimal X-ray evidence of osteoporosis. Other subjects with osteoporosis also responded by positive change in calcium balance and decreased rate of excretion of calcium in urine. (In one, with osteoporosis secondary to treatment of rheumatoid arthritis with prednisone, urinary calcium excretion fell from 204–240 mgm. per day to 24–68 mgm. per day from the twelfth to the fifteenth week of treatment. This was accompanied by increase of about 80 mgm. a day of fæcal calcium; the intake remaining constant.)

Other significant changes were, in several osteoporotic patients, increased alkaline phosphatase activity in plasma, from normal pre-treatment values to 16–38 King–Armstrong units per 100 ml., decreased rate of excretion of citrate and accelerated excretion of acid mucopolysaccharides in urine. No significant alteration occurred, during treatment of any patient, of concentration in plasma of calcium, phosphorus or citrate. No change in X-ray appearance of the skeleton or abnormality of hæmatological, renal or hepatic function could be measured. The only evidence of possible untoward effect was that, during the period of treatment, two of the seven subjects developed sub-acromial bursitis.

Although these results do not show whether or not fluoride will be useful in treatment of human subjects, they do demonstrate profound effects of fluoride ion on calcium metabolism of patients with several skeletal diseases.

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PHARMACOLOGY

Effect of Serotonin on Absorption and Distribution of Calcium-45 in the Rat

CERTAIN pharmacological effects of serotonin might be related to its action on the permeability of cell membranes to various ions. It has been shown that serotonin inhibits the pigment effusion, sodium uptake and potassium efflux from slices of red beetroots *in vitro*[1]. The loss of potassium from erythrocytes in cold storage is reduced by serotonin[2]. An increase of the efflux of sodium and potassium from the isolated skin of a frog was also shown in the presence of serotonin[3]. This latter effect was not considered to be the result of non-specific damage of the cell membranes, because the permeability to Evans blue or sucrose was not affected by serotonin[3]. It is, however, well known that serotonin exerts a marked effect on the capillary permeability in rats[4–6]. Following a different approach, Woolley has proposed that there is an interaction between serotonin and calcium in isolated organs on the basis that serotonin no longer stimulates the uterus in the presence of a calcium-chelating agent[7]. This finding was interpreted as an effect of serotonin in facilitating the penetration of calcium into the cells[8]. On the other hand, calcium seems to be an important factor for serotonin release during the antigen–antibody reaction[9,10].

These results prompted us to investigate a possible role of serotonin in the absorption and distribution of calcium. Experiments have been carried out *in vitro* on the everted intestinal sac of the rat, according to the method of Wilson and Wiseman[11]. Serotonin (5-hydroxytryptamine creatinine sulphate) at a concentration of 20 μgm./ml. increased the absorption of calcium-45 (after 15, 30 or 60 min. of incubation), while acetylcholine (20 μgm./ml.) had no effect. Creatinine sulphate (20 μgm./ml.) was also ineffective. Serotonin retained its effect when the everted intestinal sac was incubated at 5° C. Some of the results are summarized in Table 1.

Table 1. Effect of Serotonin and Acetylcholine on Calcium-45 Absorption from the Everted Intestinal Sac after 30 min. of Incubation

Substance added		Per cent of calcium-45 absorbed*	Significance (P)
—			
None		4·14 ± 0·09	—
Serotonin	10	5·49 ± 0·16	< 0·01
Acetylcholine	10	3·45 ± 0·06	= 0·01
None		4·26 ± 0·11	—
Serotonin	20	8·05 ± 0·13	< 0·01
Acetylcholine	20	3·67 ± 0·10	> 0·05

*Each value is the mean of 8 determinations.

The everted intestinal sac (10 cm.) was suspended in 5 ml. of medium containing: NaCl 0·9 per cent (100), KCl 1·15 per cent (4), KH_2PO_4 2·11 per cent (1), buffer phosphate at pH 7·4 (10), glucose 0·3 per cent in the final solution. 1 ml. of the same medium was introduced inside the sac. $^{45}CaCl_2$ was added to the outside medium at the dose of 0·4 μc./ml. (specific activity 2·5 μc./mgm. calcium). The flasks were incubated in O_2 at 37° C. in a Warburg apparatus at 96 oscillations/min.

The values represent the percentage of the added radioactivity recovered 'inside' the sac.

The effect of serotonin does not seem to be related to the contraction of the intestine, because acetylcholine at the same concentration is ineffective. Serotonin also increased the penetration of calcium into human cancer cells (strain $Af(H.Ep2)$)[12] cultivated *in vitro*, as shown in this laboratory by autoradiography (A. Leonardi and R. Vertua, unpublished observations).

Further experiments were carried out to see if the serotonin effect also occurs *in vivo*. Male Sprague Dawley rats, 150 gm. weight, were fasted for 6 hr. and then given both serotonin and calcium, simultaneously, by various routes. The animals were killed at different intervals of time afterwards.

Samples of liver (3 gm.), brain (1 gm.), washed small intestine (2·5 gm.) and blood (1 ml.) were combusted for 12 hr. at 200° C. and for an additional 24 hr. at 700° C.[13]. The ashes were dissolved in 2 N hydrochloric acid and mixed with 6·45 mgm. of calcium chloride as carrier. Ammonium oxalate, 4 per cent, was added in excess and then neutralized with ammonia, 25 per cent. When calcium oxalate was completely precipitated (after 4 hr.) it was centrifuged and washed several times with ammonia, 2 per cent, and distilled water. The sample was then plated and the radioactivity was measured using a thin mica end-window Geiger counter.

Table 2. Effect of Serotonin on Distribution of Calcium-45

Serotonin mgm./kgm.	hr. between calcium uptake and killing	c.p.m./mgm. calcium			
		Blood	Small intestine	Liver	Brain
—	2	1,023	1,878	1,362	321
25 oral	2	1,230	3,529	1,935	376
—	4	1,153	2,075	703	326
25 oral	4	1,213	2,397	1,120	344
—	1	2,240	9,432	1,519	191
10 i.p.	1	3,824	30,114	1,948	212
—	2	1,572	3,848	1,143	260
10 i.p.	2	2,974	17,816	3,349	390
—	4	1,405	2,730	1,170	378
10 i.p.	4	1,822	5,227	1,903	380

$^{45}CaCl_2$ (specific activity 2·5 μc./mgm. calcium) was given by oral route at the dose of 150 μc./kgm.

It is evident from Table 2 that serotonin increases the absorption of calcium from the intestinal tract. In other experiments not reported in detail, the distribution of calcium-45 after intravenous administration was not greatly affected by serotonin. For this reason, it is likely that the increase of calcium in liver, when calcium was given orally, should probably be related to the increased absorption of calcium from the intestine.

The pharmacological implications of these observations are now under investigation.

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Potentiation of Epinephrine Action

The following experimental results have been reported by our colleagues on the potentiation of epinephrine action by various substances, clarifying part of its mechanism: (1) The potentiation of epinephrine action on vasoconstriction by certain amino-acids was attributed to a reaction in which the oxidized form of epiaephrine gave rise to the oxidative de-amination of amino-acids and was reduced by the generated hydrogen[1,2]. (2) The epinephrine potentiation in the presence of histidine resulted from its combination with cupric ion in the tissues, which was considered as one of the oxidizing catalysts of epinephrine[3].

On the other hand, it is generally accepted that there exist some kinds of enzymes which attack epinephrine in living organisms. To investigate the influence of the so-called epinephrine-potentiating substances such as cocaine, ephedrine and catechol-derivatives on these enzymes, O-methyl transferase[4] especially has caught our interest. We have carried out a series of experiments to evaluate the epinephrine potentiation which is attributable to the inhibition of monoamine oxidase and O-methyl transferase by these substances. The following results have been obtained: (1) The inhibitory strength of monoamine oxidase inhibitors such as cocaine, ephedrine, procaine, marsilid and methylene blue *in vitro* did not parallel the degree of epinephrine-potentiating action upon vasoconstriction[5]. (2) Cocaine, ephedrine and procaine manifested no influence on O-methyl transferase activity. Pyrogallol, gallic acid, catechol, some catechol-derivatives and flavonoids proved to be the substrates of O-methyl transferase, and these substances, when co-existing with epinephrine, competitively inhibited the metanephrine formation (Table 1). (3) The epinephrine potentiation by hydroquinone[6] was observed to have no relation to the change in the activity of O-methyl transferase, but it seemed more likely that the reduction of Fe^{+++}-protein followed by the increment of Fe^{++}-protein, a probable constituent of epinephrine receptor[7,8], was the main cause.

Table 1. Inhibition of Various Substances on Metanephrine Formation

Substance	Dose (μmole)	Metanephrine formed (μmole)
Control	—	$4{\cdot}7 \times 10^{-2}$
Cocaine	2·0	$4{\cdot}8 \times 10^{-2}$
Ephedrine	2·0	$4{\cdot}2 \times 10^{-2}$
Procaine	2·0	$4{\cdot}8 \times 10^{-2}$
Catechol	0·5	$1{\cdot}3 \times 10^{-2}$
Protocatechu aldehyde	0·5	0
Protocatechuic acid	0·5	$0{\cdot}7 \times 10^{-2}$
3,4-dihydroxy phenylacetic acid	0·5	$2{\cdot}1 \times 10^{-2}$
Hydroquinone*	0·5	$4{\cdot}9 \times 10^{-2}$
Gentisic acid*	0·5	$4{\cdot}9 \times 10^{-2}$
Pyrogallol	0·5	0
Gallic acid	0·5	0
Phloroglucin	0·5	$4{\cdot}2 \times 10^{-2}$
Rutin	0·5	0
Quercetin	0·5	0

Each substance was added to the following mixture and incubated at 37·5° C. for 30 min. under anaerobic conditions: epinephrine, 0·5 μmole; magnesium chloride, 20 μmole; *S*-adenosylmethionine, 0·5 μmole; *Iris*-buffer, 100 μmole; crude enzyme (the soluble supernatant fraction obtained from rat liver prepared by Axelrod's method (ref. 4)), 0·6 ml. and water to make a final volume of 2·0 ml. Metanephrine formed was estimated by Pisano's method. Each value is the mean of three experiments. The standard deviation was within 10 per cent.

* Gentisic acid and hydroquinone proved to be the substrates of O-methyl transferase in this reaction mixture.

Table 2. Inhibition of the Uptake of Epinephrine by Cocaine, Ephedrine and Procaine

	Dose (final concentration, *M*)	Epinephrine uptake (μgm./mgm. of dry weight)
Control (0° C.)	—	0·35
Control (37·5° C.)	—	3·30
Ephedrine	10^{-3}	0·48
Cocaine	10^{-3}	0·51
	10^{-4}	0·73
	4×10^{-5}	0·99
	4×10^{-6}	1·99
	4×10^{-7}	2·64
Procaine	4×10^{-5}	2·79
	4×10^{-6}	3·06
	4×10^{-7}	3·10

Platelets were suspended in 2·5 ml. of the phosphate saline containing 200 μgm. of epinephrine and different concentrations of each substance. The mixture was incubated at 37·5° C. for 90 min. Values given represent the mean of three experiments. The standard deviation was within 10 per cent.

Recently, problems on the uptake of catecholamines by some tissues and release from them have become of interest to us. It is a matter of course that the mechanism of epinephrine potentiation should also be analysed from this point of view. Accordingly, we have tried a model experiment on the uptake and release of epinephrine using rabbit blood platelets, and obtained the following result: The uptake of epinephrine by rabbit blood platelets was inhibited by cocaine, ephedrine and procaine (Table 2), and their inhibitory strength was parallel with their potentiation degree of epinephrine action.

From our experimental results mentioned here, we have classified the mechanism of the epinephrine potentiation by various substances as follows:

(1) Competitive inhibition on the enzymes, especially *O*-methyl transferase in inactivating epinephrine. (Pyrogallol, gallic acid, catechol, some catechol-derivatives, and flavonoids.)

(2) Inhibition of the uptake of epinephrine by blood platelets, blood cells and other tissues. (Cocaine, ephedrine and procaine.)

(3) Chelation with cupric ion that is a catalytic agent of the oxidation of the phenol ring of epinephrine. (Histidine, alanine, glycine, methionine and phenylalanine.)

(4) Acceleration of 'oxidation-reduction' turn-over of epinephrine. (Certain amino-acids and ascorbic acid.)

(5) Reduction of Fe^{+++}-protein to Fe^{++}-protein, a probable constituent of epinephrine receptor. (Hydroquinone, cysteine, ascorbic acid and glutathione.)

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HÆMATOLOGY

Action of Influenza Viruses, Receptor-destroying Enzyme and Proteases on Blood Group Agglutinogen M^g

The ability of influenza viruses and receptor-destroying enzyme from *Vibrio cholerae* to inactivate human erythrocyte agglutinogens *M* and *N* was first noted in this Laboratory[1] and afterwards independently in Finland[2]. Lu^a and Lu^b agglutinogens are also inactivated by influenza viruses but not by the receptor-destroying enzyme[3]. None of 17 additional human blood group agglutinogens—including *S* and *s* which are considered to be products of genes very closely linked to the *M* and *N* genes—was destroyed by these agents[1,3]. On the other hand, the inactivation of a number of erythrocyte agglutinogens including *M* and *N* by papain, trypsin and other enzymes has been known for a considerable time[4–6].

Recently a new blood-group factor, M^g, was described by Allen, Corcoran, Kenton and Breare[7] and thought to be the product of a gene allelomorphous to the *M* and *N* genes. It was of interest, therefore, to investigate the effect of enzymically active agents on this 'new' erythrocyte agglutinogen.

B, MM^g and *O*, NM^g erythrocytes (Paul and John Gilfeather respectively; cf. ref. 7) and serum (lot No. SM^g-50) containing saline agglutinating anti-M^g antibodies of a titre of 1 : 8 were obtained with the co-operation of Dr. F. H. Allen, jun., from the Blood Grouping Laboratory, Boston. A saline-agglutinating anti-M^g serum with a titre of 1 : 1–1 : 2, found in this Hospital's blood bank, was also used. The erythrocytes were washed with 0·85 per cent saline and treated with purified influenza viruses (Melbourne and Swine S_{15} of type *A* and Lee of type *B*), with the receptor-destroying enzyme from *Vibrio cholerae*, papain and trypsin as previously described[1,3] and also with three times the amount of virus used in earlier experiments. Physiological saline buffered with 0·005 *M* veronal to *p*H 7·4 and containing a trace of calcium chloride was substituted for the phosphate buffer used earlier.

Table 1. EFFECT OF INFLUENZA VIRUSES AND RECEPTOR-DESTROYING ENZYME ON HUMAN ERYTHROCYTE AGGLUTINOGENS (GILFEATHER)

Serum		Agglutinogen tested for	Erythrocytes	Serum unabsorbed: Agglutination of erythrocytes* Native	Serum unabsorbed: Agglutination of erythrocytes* Neuraminidase-treated	Serum absorbed with neuraminidase-treated erythrocytes: Agglutination of erythrocytes* Native	Serum absorbed with neuraminidase-treated erythrocytes: Agglutination of erythrocytes* Neuraminidase-treated
anti-*M*	Human (*J.C.*)	*M*	Paul Gilfeather *B*, *MM^g*	8	4–8	8	<1
	Human (*SJM*–1)	*M*		4–8	1	4	<1
anti-*M^g*	Human (*SM^g*–50)	*M^g*		4–8	16	<1	<1
anti-*B*	Human (*P.W.*)	*B*		64	128	<1	<1
anti-*N*	Human (Ar. donated by Dr. H. Gershowitz)	*N*	John Gilfeather *O*, *NM^g*	32	16	16–32	<1
	Vicia graminea (*J.C.* 60)	*N*		16	<1	32	<1
anti-*M^g*	Human (*SM^g*–50)	*M^g*		8	32	<1	<1
	Human (2111/61)	*M^g*		1–2	4–8		
anti-*H*(*O*)	Eel (*E.D.*60)	*O*		128	512	2	8

* Expressed as reciprocal of serum dilution.

The results with influenza viruses and the receptor-destroying enzyme were similar and are listed in Table 1. These results show clearly that treatment of Gilfeather erythrocytes with influenza viruses or the receptor-destroying enzyme inactivates *M* and *N* but not *M^g* (columns 3 and 4, Table 1). The *B* and *H*(*O*) antigens of these erythrocytes, serving as controls, are not inactivated by this treatment. Lack of inactivation of the *M^g*, *B* and *O*(*H*) agglutinogens is demonstrated by the increased titres of the respective agglutinins (columns 3 and 4, Table 1) against neuraminidase-treated erythrocytes as compared to the titres against native erythrocytes. The increase in agglutinin titre against non-inactivated agglutinogens appears to be due to the additive effect of the titre against the native receptor plus *T*-agglutination against the *T* antigen unmasked by neuraminidase (cf. ref. 1). The *T* agglutinin alone, however, is not sufficiently strong to prevent the demonstration of the inactivation of the *M* and *N* factors as the agglutinin titre does not rise but decreases (column 4, Table 1). To eliminate the interfering action of *T* agglutinins, erythrocytes treated with the same enzymically active agent were used for both absorption of sera and titration of agglutinins. A comparison of the data in the third and fifth columns of Table 1 shows that absorption with neuraminidase-treated erythrocytes removes anti-*M^g*, anti-*B* and anti-*H*(*O*) agglutinins but not anti-*M* and anti-*N* agglutinins. The anti-*M* and anti-*N* titres against untreated erythrocytes are the same before and after absorption. This indicates inactivation of *M* and *N* agglutinogens of the neuraminidase-treated red cells, as does also a comparison of the last two columns of Table 1, where the titres are listed after removal of anti-*T* agglutinins. Trypsin and papain, however, completely inactivate the *M^g* receptor. Untreated *M* and *N* erythrocytes removed their respective antibodies in one absorption with one volume of packed erythrocytes.

There was no demonstrable change of the agglutinogens under investigation when they were incubated with either uninoculated allantoic fluid, boiled influenza viruses or autoclaved enzymes.

B, *MM^g* and *O*, *NM^g* erythrocytes were agglutinated by influenza viruses as were other human erythrocytes and also became inagglutinable by the virus to which they had been exposed extensively.

The lack of inactivation of agglutinogen *M^g* by influenza viruses and the receptor-destroying enzyme, in contrast to that of antigens *M* and *N*, is unexpected in view of the assumed close serological and genetical relation of blood group receptors *M*, *N* and *M^g* (ref. 7). It recalls, however, similar observations for the *ABO* blood-group system where certain enzyme preparations will inactivate all three blood group antigens while others will affect only one. It is believed that influenza viruses possess only one enzyme, neuraminidase[8]. Based on the activity of influenza viruses, it has been assumed that derivatives of neuraminic acid (sialic acids) are closely involved in the specificity of blood group agglutinogens *M* and *N*[1,2,10,11]. While this assumption is reasonable, it is obvious from the data presented here that if *M^g* is indeed an allele to *M* and *N*, it is unlikely that identical sialic acids in identical linkages are involved in the specificities of the products of the allelomorphic genes *M*, *M^g* and *N*. Influenza virus enzyme may thus be a more sensitive tool than papain and trypsin in the investigation of fine structural differences of products of blood-group genes of the *MNS* system.

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The Diego Factor in a Puerto Rican Family: a Case of Anti-Diego in Monozygotic Twins

THE Diego blood factor exists in many South American people having a substantial Indian admixture[1]. The following family is the first reported from Puerto Rico.

Mrs. P. F. M., a Puerto Rican woman, age thirty-seven, residing in New York City, delivered premature, monozygotic, female twins, with positive direct Coombs tests. She had four full-term spontaneous

deliveries by her first husband (*Dia*-), and two early abortions and one miscarriage before the twins, by her second husband (*Dia*+). On close observation for seven weeks neither twin developed any signs or symptoms of hæmolytic disease of the new-born other than minimal hepato-splenomegaly.

Serological investigations revealed the father to be group *O*, R_1R_1, *Dia*+, both twins to be group *A*, *Dia*+, R_1R_2, the mother, group A_2, R_2r and *Dia*-. The hæmolytic antibody formed by iso-immunization was anti-Diego, detected by an indirect Coombs test with a titre 1 : 32. Investigation of the father's family revealed the Diego factor in his aunt, paternal grandmother, and great uncle, all natives of Puerto Rico and exhibiting Indian features. The *Dia*+ cases were typed for *ABO*, *D*, *C*, *c*, *E*, *e*, *V*, *MNSs*, *P*, *K*, *k*, *Fya*, *Lea*, *Leb*, *Jka*, *Jkb*, *Lua*, *Lub*, *Jsa*, *Yta*, *Kpb*, *Wra*, Vel, Good, Santos, Gerbich, without adding new information pertaining to the independent segregation of *Dia* gene.

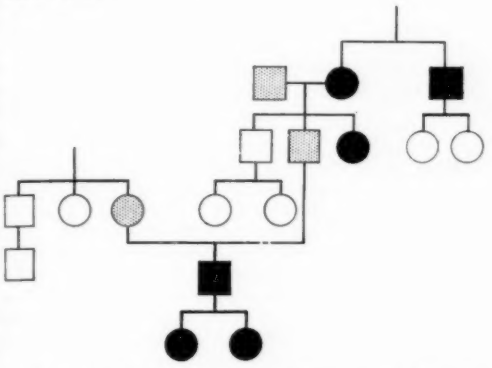

Fig. 1. The anti-Diego (*Dia*) factor in four generations of a Puerto Rican family. Black, *Dia*-positive; shaded, not tested, deceased; open, *Dia*-negative

This anti-Diego is the first reported which caused no symptoms in the infants who provoked immunization. The prematurity of these twins should have heightened their susceptibility to hæmolytic disease. Perhaps several near-term gestations are necessary to stimulate formation of a clinically apparent antibody, a state analogous to Rh disease in non-transfused patients.

The original case (Diego), a Venezuelan child, who died of hæmolytic disease of the new-born, was fourth in birth order with two older *Dia*+ siblings[2]. The second case (W.O.J.) was third in birth order with non-fatal hæmolytic disease (Levine, P., personal communication). The third case was fifth in birth order (Mart) and died from severe erythroblastosis fœtalis[3].

Table 1

	Mother	Twins	Father
Group	A_2	*A*	*O*
Rh D	+	+	+
C	−	+	+
E	+	+	−
d	+	−	−
c	+	+	−
e	+	+	+
	cDE/cde	*DCeDcE*	*DCe/DCe*
WRa	−	−	−
Kpa	−	−	−
Kpb	+	+	+
He	−	−	−
Dia	−	+	+

The true hazard of sensitization to *Dia* is not known. Layrisse stated that in 50 cases of *Dia* incompatible matings no antibodies were formed in an unspecified number of gestations[4].

Among Chinese with a 2·5 per cent incidence of *Dia*, hæmolytic disease is very rare. Clinically, anti-Diego has not yet proved to be of great significance. Nevertheless, the present case demonstrates that undetected antibodies to *Dia* will be missed if not sought for. Our rule of an antiglobulin test on premature infants revealed iso-immunization. Among populations with a high incidence of the Diego factor, it remains a potential cause of fœtal wastage.

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PATHOLOGY

Effects of Carrageenin on Serum Lipids and Atherosclerosis in Rabbits

In recent years considerable interest has developed regarding an important role of aortic mucopolysaccharides with advancing age. On the other hand, a number of reports have dealt with the activities of heparin and other sulphated polysaccharides to clear visible lipæmia and also to retard arteriosclerotic process in arterial walls.

The object of this communication is to report a hitherto undescribed effect of carrageenin, a natural sulphated polysaccharide, obtained from seaweeds, in suppressing hyperlipæmia and atherosclerosis in cholesterol-fed rabbits. Carrageenin obtained from *Chondrus ocellatus* contained 11·86 per cent sulphur. It showed a specific optical rotation of +28°. The experiment was performed as follows :

A number of male albino rabbits were all fed a daily diet consisting of bean-curd-mash and cholesterol at the rate of 100 mgm. per kgm. body-weight during the course of 18 weeks. The animals were divided into two groups : (1) cholesterol-fed plus carrageenin-injected group consisted of 21 rabbits ; (2) a control group of 19 rabbits fed cholesterol only. The animals of the treated group received individually a daily intravenous injection of 5 mgm. of carrageenin per kgm. body-weight. At 2-week intervals, serum lipids such as total lipid[1], total and esterified cholesterol[2], phospholipid[3], β/α lipoprotein ratio[4] and turbidity of the serum were measured. At the end of the experiment, all rabbits were killed, and the atheromatous lesions in the aorta were examined macroscopically and microscopically.

The results of the experiment revealed that carrageenin possesses suppressing effects on the rise of

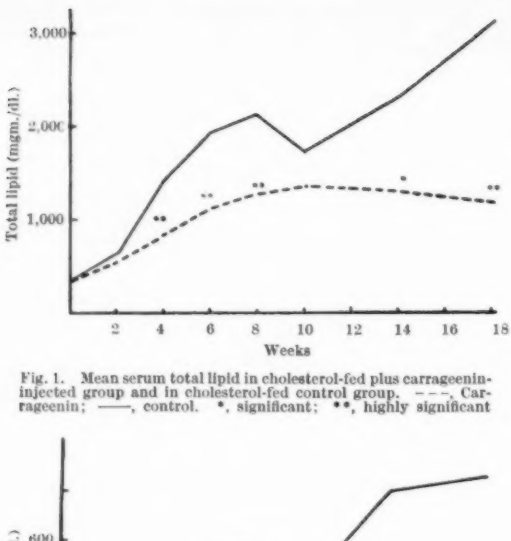

Fig. 1. Mean serum total lipid in cholesterol-fed plus carrageenin-injected group and in cholesterol-fed control group. – – –, Carrageenin; ——, control. *, significant; **, highly significant

Fig. 2. Effect of carrageenin on serum total cholesterol. – – –, carrageenin; ——, control. *, significant; **, highly significant

serum lipids in cholesterol-fed rabbits. Figs. 1 and 2 show that the mean serum total lipid and total cholesterol-levels in the treated group showed less increase compared with the control after 4 weeks following the beginning of injection of carrageenin, and significant differences between the two groups were noticed during the experiment except for the early period. In addition, remarkable differences of the mean levels in cholesterol and phospholipid ratio, β/α lipoprotein ratio and serum turbidity in both the groups were noticed. Lipæmia-clearing activity was shown following the administration of carrageenin. However, carrageenin failed to prove its anticoagulant activity, at least at the dosage of 5 mgm. per kgm. body-weight, according to the Lee and White method.

The visual grading of severity of atheromatous lesions, as determined by their distribution, size and number, was shown to be almost parallel to the concentration of the serum lipids. The microscopic findings, according to several stainings, supported the result in the gross findings that the grade of severity of atheromatous lesions was much less in the carrageenin-treated rabbits than in the controls.

T. Mori[5] reported that carrageenin consists of 30–40 per cent D-galactose and 20–30 per cent sulphate. The finding[6] that the subcutaneous injection of carrageenin into animals stimulated the formation of new connective tissue will support the presumption that carrageenin plays a part in promoting the repair of atheromatous lesions, when an adequate supply of it is provided.

Grateful acknowledgment is made to Prof. Y. Oshima for his kind advice while carrying out this investigation.

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Immunofluorescence of Pleuropneumonia-like Organisms isolated from Tissue Cell Cultures

It has been established that continuous tissue cell cultures are commonly contaminated with pleuropneumonia-like organisms and/or *L*-forms of bacteria[1-5]. These results have encouraged investigators to search for reasonably simple methods for the detection of these agents, especially in relation to studies for their elimination. Of methods proposed for their detection, cultivation and isolation have proved most successful[6]. With the advent of the fluorescent antibody technique as a rapid method for the identification of micro-organisms, it seemed to us that this procedure might be useful as a means of detecting contamination of pleuropneumonia-like organisms in tissue cell cultures. The feasibility of this technique is presented here.

Strain *HEp* 2, isolated from the human epidermoid carcinoma of skin (Fjelde) cell line, was propagated in primary rabbit kidney cells for the preparation of antigen for antibody production in New Zealand white rabbits. The antiserum was conjugated with fluorescein *iso*thiocyanate in the manner described by Marshall *et al.*[7]. Lissamine rhodamine *RB* 200 was conjugated to normal bovine serum albumin and added to the labelled antiserum as a counterstain[8].

Contaminated continuous cell cultures and infected primary rabbit kidney cells grown on cover-slips in Leighton tubes were fixed in buffered formalin, washed, and then stained with the fluorescein-conjugated antiserum for 1 hr. at 37° C. in a wet chamber.

The strains of pleuropneumonia-like organisms, isolated from the cell lines mentioned here, were also grown on membrane disks (Millipore Filter Corporation, Bedford, Massachusetts) placed on PPLO agar medium[6]. Impressions were made by transferring colonies from disks to microscopic slides, air drying, and staining them for 15 min. at room temperature. The stained preparations were examined with a Zeiss fluorescence microscope, using the Osram *HBO* 200 light source, the *BG*-12 exciter filter, and the *GG*4-*OG*4 barrier filter.

Fluorescein-conjugated antiserum reacted specifically with the homologous pleuropneumonia strain (Fig. 1) when tested with the original contaminated cell lines, infected primary cells, and colonial impres-

sions. Strains isolated from five other continuous cell cultures maintained regularly in both of our laboratories reacted in the same manner. These cultures were HeLa—human carcinoma of cervix (Gey); HA—human amnion (Fogh and Lund); HI—human embryonic intestine (Henle); KB—human carcinoma of nasopharynx (Eagle); and conjunctiva—human adult (Chang). Non-infected primary tissue cell cultures previously found by cultural methods to be free of pleuropneumonia-like organisms gave no staining reactions. These preliminary findings suggest that tissue culture strains of these organisms may have similar antigenic structures. Since the organisms were isolated from tissue cultures used in different laboratories, the chance of contamination from a common source could probably be ruled out. On the other hand, it may be that tissue cell cultures favour the selection and development of the same or related strains of the organism.

Heterologous human urethral strain 07 (Dr. Paul Smith, Philadelphia) and strain Campo (Dr. Louis Dienes, Boston) did not react with the *HEp* 2 tissue culture antiserum. This indicates that there are differences between the antigenic structures of these strains. The reaction and non-reaction of colonial impressions of *HEp* 2 and 07, tested with the antiserum simultaneously on the same slide, are shown in Fig. 2.

In order to establish the specificity of the fluorescein-conjugated antiserum of the organisms, 24 bacterial and mycotic organisms were tested. None reacted. Included were strains from the genera *Salmonella*, *Shigella*, *Proteus*, *Staphylococcus*, *Streptococcus*, *Diplococcus*, *Corynebacterium*, *Pseudomonas*, *Serratia*, *Escherichia*, *Aerobacter*, and *Cryptococcus*.

Antisera to vaccinia, adenovirus type 7, herpes simplex, and infectious canine hepatitis viruses prepared in rabbits and conjugated with fluorescein isothiocyanate did not react with the six continuous cell cultures contaminated with the organism.

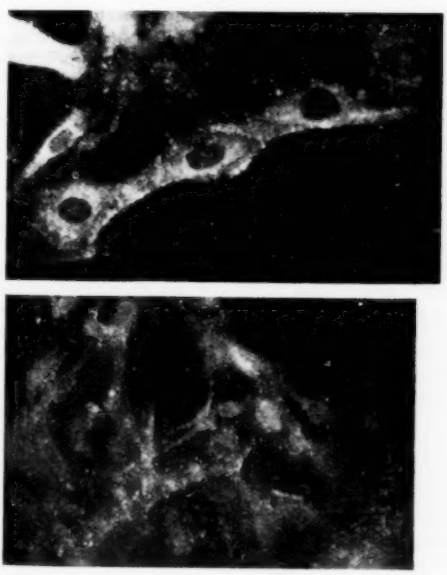

Fig. 1. Monkey (*a*) and rabbit (*b*) kidney tissue cells infected with pleuropneumonial strain *HEp* 2 stained with labelled homologous immune serum. (*a*, × 225; *b*, × 200)

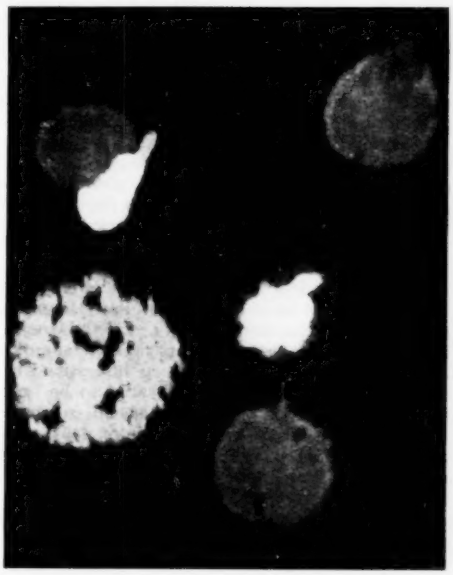

Fig. 2. Impression smears of pleuropneumonial strains *HEp* 2 and 07 stained with labelled *HEp* 2 immune serum. (× 270)

Recent reports indicate that *L*-forms of bacteria rather than pleuropneumonial organisms are the responsible contaminants of cell cultures. Workers in the United States[8] have identified a Gram-negative rod as the bacterial parent, whereas workers in Great Britain[5] have reported a *Corynebacterium*. If the organisms discussed in this communication were in fact *L*-forms of bacteria, then there is reason to suggest that a common bacterium was the source or that the *L*-forms of at least some heterologous bacteria have common antigens.

The staining pattern of fluorescence of pleuropneumonial organisms in tissue cell cultures is similar to the intracytoplasmic granulation pattern consistent with contamination by pleuropneumonial organisms. The fluorescent bodies were found concentrated along the cytoplasmic membrane, in the cytoplasma, and at times along the nuclear membrane. They were not seen within the nucleus.

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IMMUNOLOGY

An Immunological Study of a Human Pituitary Interstitial Cell-stimulating Hormone

SEVERAL investigators[1–5] have reported recently the production of rabbit antiserum to human urinary gonadotrophins, but no similar data have appeared with respect to human pituitary gonad-stimulating substances. However, an immunochemical investigation in this Laboratory[6] has shown that a highly purified preparation of sheep pituitary interstitial cell-stimulating hormone (ICSH) is a potent antigen. The rabbit antiserum obtained in that work[6] was highly specific for the antigen and was able significantly to counteract its ability to stimulate the ventral prostate in hypophysectomized rats. By the Ouchterlony double diffusion test in agar-gel and the quantitative precipitin technique, the sheep interstitial cell-stimulating hormone was demonstrated to be homogeneous. Moreover, antiserum to sheep interstitial cell-stimulating hormone was capable of neutralizing significantly the interstitial cell-stimulating hormone activity of pituitary extracts from the rat, pig and whale, as well as of purified preparations of human pituitary interstitial cell-stimulating hormone and pregnant mare serum gonadotrophin. Injected antiserum to sheep interstitial cell-stimulating hormone was also shown to cause an inhibition of endogenous activity of the hormone in normal rats. The results of similar investigations on the antigenicity of human pituitary interstitial cell-stimulating hormone are reported here.

The antigen, human interstitial cell-stimulating hormone, was prepared from human pituitaries by the procedure previously described[7]. Two albino rabbits weighing initially 2·6–2·8 kgm. were immunized with a total dose of 8 mgm. of the purified hormone per rabbit, according to the following schedule. Each rabbit was given three injections, consisting of 1·5 mgm. of human interstitial cell-stimulating hormone in Freund's adjuvant (complete), once weekly for three consecutive weeks, was allowed to rest for ten days, and was then given a fourth injection of 1·5 mgm. of human interstitial cell-stimulating hormone. The animals were once again rested for ten days, during which time they were usually test-bled from the marginal ear vein. Finally, animals were bled by heart puncture ten days after a booster shot of 2 mgm. of human interstitial cell-stimulating hormone in saline. The pooled sera showed the presence of contaminating antibodies to normal human serum proteins and, to a very slight extent, to human growth hormone. These contaminating antibodies were selectively removed by absorption by the addition of small quantities of 1 : 10 diluted normal human serum and a dilute solution of human growth hormone. The agar-gel diffusion test was used to determine whether the absorption was complete. This absorbed serum was used in all subsequent experiments.

The presence in the absorbed antiserum of a precipitating type of antibody specific to human interstitial cell-stimulating hormone was demonstrated by means of the qualitative ring test[8], double diffusion on Ouchterlony agar-gel plates[9], immunoelectrophoresis[10], and on the basis of antihormone activity as measured by the capacity of the antiserum to neutralize the activity of the test material in hypophysectomized male rats[11,12]. The capacity of antiserum to human interstitial cell-stimulating hormone to neutralize the biological activity of human chorionic gonadotrophin (HCG) was also tested in the normal female rat.

In the qualitative precipitin ring test, by layering 0·1 ml. of absorbed antiserum under a solution of antigen, it was possible to detect 1 μgm. of human interstitial cell-stimulating hormone. The single precipitin line which human interstitial cell-stimulating hormone gave with the absorbed antiserum in both agar-gel double diffusion analysis and immunoelectrophoresis demonstrated the homogeneity of the antigen–antibody system. It was further noted that the antiserum cross-reacted with a component (interstitial cell-stimulating hormone) in a saline extract of human pituitary glands, giving a single continuous precipitin band with that formed with purified human interstitial cell-stimulating hormone in the agar-gel double diffusion experiment.

Table 1 presents results concerning the capacity of the human interstitial cell-stimulating hormone antiserum to inhibit or neutralize effectively the activity of human gonadotrophins in hypophysectomized male rats; a total dose of 1·0 ml. of the antiserum given over a period of 4 days effectively neutralizes the biological activity of human interstitial cell-stimulating hormone and human chorionic gonadotrophin.

In normal female rats, the biological activity of human chorionic gonadotrophin was counteracted by the simultaneous administration of rabbit antiserum to human interstitial cell-stimulating hormone; when human chorionic gonadotrophin alone was given to a group of 10 rats (21 days of age at onset), a total dose of 40 μgm. administered intraperitoneally over a period of 3 days produced an ovarian weight (mean ± standard error) of 36·2 ± 1·92 mgm., whereas with the same dose of human chorionic gonadotrophin plus a 1-ml. total dose of the antiserum the weight of the ovaries was only 15·5 ± 1·09 mgm. Furthermore, when tested by the Ouchterlony double diffusion technique, human chorionic gonadotrophin cross-reacted with rabbit antiserum to human interstitial cell-stimulating hormone. It can thus be concluded that human interstitial cell-stimulating hormone is a potent antigen and that it should be possible to develop immunochemical assay methods to determine the content of interstitial cell-stimulating hormone in human pituitary gland and in body fluids.

Table 1. EFFECT OF RABBIT ANTISERUM TO HUMAN INTERSTITIAL CELL-STIMULATING HORMONE ON THE BIOLOGICAL ACTIVITY OF GONADOTROPHIN PREPARATIONS AS ASSAYED IN IMMATURE HYPOPHYSECTOMIZED MALE RATS

Group	No. of rats per group	Material assayed	Total dose in 4 days (μgm. + ml.)	Weight of ventral prostate (mgm.)	Statistical comparison of groups
1	9	Uninjected controls	0	7·0 ± 0·14*	
2	15	Human ICSH	8 + 0	18·1 ± 0·49	
3	7	Human ICSH + antiserum	8 + 1	7·5 ± 0·30	2, 3; $P < 0.001$†
4	6	HCG	1 + 0	15·5 ± 1·5	
5	5	HCG + antiserum	1 + 1	10·0 ± 0·83	4, 5: $P < 0.01$

* Mean ± standard error. † Values calculated according to Fisher's *t*-test.

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Occurrence of an *Rh*-like Antigen in Red Cells of the Sheep

Landsteiner and Wiener[1] discovered that the sera of rabbits immunized by the erythrocytes of the rhesus (macacus) monkey would agglutinate those of certain human subjects irrespective of their *ABO* groups. They added, therefore, the *Rh*-antigen to the list of antigens characterizing the human red cells. Some human red cells, therefore, must be assumed to possess a receptor-substance for antibodies which rabbits or still better guinea pigs can form when immunized with rhesus erythrocytes. Later, Wiener and Peters[2] demonstrated the antigenicity of the *Rh*-receptor of human erythrocytes in man, thus establishing the principal cause for erythroblastosis in the new-born.

Apart from the *Rh*-antibody obtained by immunization of guinea pigs with rhesus erythrocytes, there are antibodies formed in women eventually immunized when bearing a fœtus possessing *Rh*-erythrocytes or possibly formed in human males and females following transfusion of blood incompatible with *Rh*.

As is known, *Rh* denotes a group of antigens distinguishable in man and the *Rh*-antigen marked *D* according to Fisher[3] is the most common in man, and human *D*-erythrocytes are also agglutinated by an anti-*Rh* guinea pig immune serum.

Therefore, two anti-*D* sera occur, one the human anti-*D* and the other an animal immune anti-*Rh* serum which can be used as anti-*D*. These sera, however, are different: the human anti-*D* serum contains only human *Rh*-antibodies, for example, anti-*D*, besides other human isohæmagglutinins of the *ABO* and other systems, while the guinea pig immunized with rhesus erythrocytes produces, besides antibodies against the rhesus *D*, those which are able to react with some receptor-substance on human erythrocytes very similar to the rhesus *D*, and when immunized with human *D*-erythrocytes the guinea pig (or rabbit) may produce besides anti-*D* also human species-specific antibodies. The latter may explain the observation[4] that animal anti-*Rh* immune serum can react with human fœtal (*Rh*-) erythrocytes. Thus, there are many differences between the animal and the human anti-*D* sera. But the anti-*D* itself appears to be different in that the rhesus *D* cannot be identical to the human *D*, since rhesus erythrocytes, while able to absorb the anti-*D* antibodies from a guinea pig anti-rhesus erythrocyte immune-serum, cannot absorb the anti-*D* from human anti-*D* serum, which itself can be absorbed easily by human *D*-erythrocytes. Even by washing three times the rhesus erythrocytes sediment which is in 1 : 1 ratio to human anti-*D* serum, we did not succeed in nine cases in decreasing the anti-*D* titre, but to one dilution step only. This failure confirms earlier observations[5] where there was no success in absorbing a guinea pig anti-rhesus erythrocytes immune serum by human *D* red blood cells.

On account of the immunizing effect, however, *D*-antigen must be present in both human and rhesus erythrocytes, but apparently in such a way masked or concealed in the stroma that its presence cannot be proved serologically, but by immunization only. Murray and Clark[6], by immunizing guinea pigs with human *dd*-erythrocytes, are believed to have succeeded in stimulating the formation of anti-*D* antibodies.

In our experiments, boiling in distilled water did not free enough *D* from rhesus erythrocytes to enable the boiled cells completely to absorb human anti-*D* antibody, but, however, to decrease its titre to one or, in some experiments, to two more dilution steps as had been possible in the experiments mentioned by using fresh rhesus red cells. Boiling red cells, however, had proved effectual in demonstrating the presence of *D* antigen in experiments with sheep erythrocytes.

When sheep red cells, suspended in distilled water (not in saline solution), are boiled, they can absorb completely anti-*D* antibodies from both anti-*D* sera from patients as well as from commercial anti-*D* test sera, while human erythrocytes, treated in the same way by boiling, were not able to absorb anti-*D* to a similar extent. A more detailed account of these experiments will be published elsewhere[7]. Here only some details may be mentioned which require further experimental research: (1) Whether the presence of the *D* antigen in the red cells of sheep bears any relation with other blood grouping characteristics in sheep is not ascertained as yet. (2) Why boiling must be done in distilled water instead of saline remains to be shown. (3) The *Rh*-antigen in sheep red cells appears to represent only that portion which is common to both *C* and *D* antigens, since the absorbing power of boiled ovine red cells, investigated so far, does not distinguish between *C* and *D* antibodies. (4) Experiments are in progress to show whether immunization of guinea pigs or rabbits with boiled red cells of the sheep will produce, besides Forssman antibodies, *Rh*-(*D*?)-antibodies.

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BIOLOGY

Experimental Evidence for the Mechanism of Photosynthesis

Five types of short-timed absorption changes (type 0, 1, 2*a*, 2*b*, 3) have been found in photosynthesis[1]. Type 0 is caused by the formation of chlorophyll-triplet states, and occurs only when conduction of energy is interrupted by altering the arrangement of chlorophyll in the chromatophores. Type 1 is caused by the formation of a chlorophyll-*a* derivative (life-time 3×10^{-5} sec.) and occurs only in the normal arrangement of chlorophyll. For details regarding types 0 and 1, see ref. 1 and especially ref. 2.

The difference spectrum of type 2 absorption change is shown in Fig. 1*a*; it appears in $\leqslant 10^{-5}$ sec. and has a life-time of $\sim 10^{-2}$ sec. Type 2 consists of two processes: type 2*a* (negative peak at $\sim$ 420 mμ) and type 2*b* (negative peak at $\sim$475 mμ and positive peak at $\sim$515 mμ). At $-150°$ C. type 2*b* does not occur; the remaining reaction is type 2*a*. From the appearance at $-150°$ C. it follows that type 2*a* must be caused by one primary product of photosynthesis. From the dependence on wave-length at $-150°$ C., it follows that the primary product is probably an oxidized cytochrome[3].

Type 2*b* change of absorption is caused by a reduction process[4]: $X0 \rightarrow X$. On extracting chloroplasts with petroleum ether, the absorption changes of type 2*b* no longer occur. They reappear on recondensation[3]. As plastoquinone is extracted by petroleum ether[5], $X/X0$ may be identical with plastoquinone. Since plastoquinone has no absorption at 515 and 475 mμ, the absorption changes probably take place indirectly by changing the absorption of other pigments during the redox reaction of $X/X0$ (ref. 1).

Since 1956 it has been known[6] that probably two chlorophyll pigments are involved in photosynthesis (Emerson effect): after Govindjee[6] one chlorophyll *a*

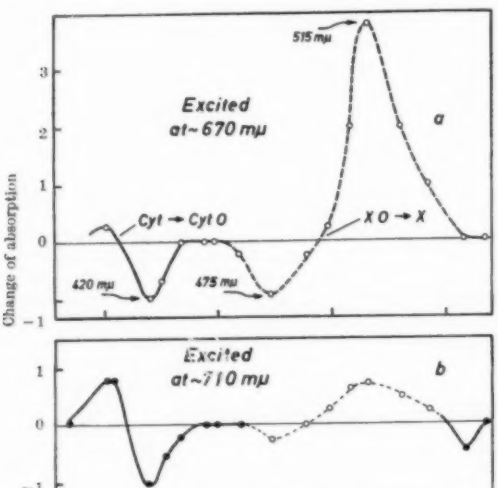

Fig. 1. Changes of absorption caused by illumination of *Chlorella* at two different wave-lengths. Temperature, 20° C.

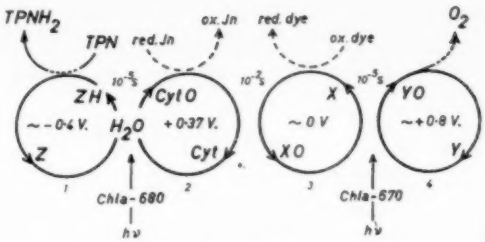

Fig. 2. Reaction pattern of photosynthesis

(*b* ?) with an absorption maximum at $\sim$ 670 mμ and a second one with a maximum at $\sim$ 680 mμ or at longer wave-lengths. We have tried to find out by which of the two pigments the primary product type 2*a* is produced and what is the function of the other pigment. Exposure at $\sim$ 670 mμ causes excitation of Chl*a*-670 and Chl*a*-680, because of the strong overlapping of the absorption bands of the two pigments. Such an excitation causes the absorption changes of type 2*a* and type 2*b*, as already shown in Fig. 1*a*. Exposure at $\sim$ 710 mμ causes mainly excitation of Chl*a*-680. The changes in absorption thus appearing are illustrated in Fig. 1*b*. Type 2*b* is reduced strongly (dotted line), whereas the absorption changes at $\sim$ 420 mμ (characteristic of type 2*a*) remain unchanged. Moreover, changes in absorption can be observed at $\sim$ 400 mμ and $\sim$ 555 mμ, which, owing to their kinetic behaviour, are to be ascribed to type 2*a* (solid line in Fig. 1*b*). These additional changes cannot be seen in Fig. 1*a*, as they are probably compensated by type 2*b*. From the dependence on wave-length of type 2*a* absorption changes (solid line in Fig. 1*b*), it follows convincingly that type 2*a* in *Chlorella* is caused at least by an oxidation of a cytochrome, probably cytochrome *f*.

These results show directly that photosynthesis is triggered by two different photochemical reactions: oxidation of cytochrome by Chl*a*-680 and reduction of $X0$ by Chl*a*-670. The occurrence of two photochemical processes results in a reaction pattern of photosynthesis which differs considerably from previous conceptions. The pattern presumed is shown in Fig. 2. ZH means the reduced form of Z (addition of H or electron); $Y0$, etc., the oxidized form of Y, etc. (loss of electron). The location of water between cycle 1 and 2 is only symbolic; the splitting probably occurs by $Y^+ + 1/2\ H_2O \rightarrow Y + 1/4\ O_2 + H^+$ and $Z^- + H^+ \rightarrow ZH$. Y and Z are chemically not known. The redox potential of cytochrome *f* is + 0·37 V. (ref. 7); that of $X/X0$ has been estimated as $\sim$ 0 V. (ref. 4). The potential of $Y/Y0$ must correspond approximately to that of OH^-/O_2 ($\sim$ + 0·8 V.). That of ZH/Z amounts to $\sim$ − 0·4 V., as triphosphopyridine nucleotide[8] and substances with redox potentials up to − 0·4 V. can be reduced by chloroplasts[9]. Thus an energy equivalent of at least $\sim$ 0·8 V. each must be produced by the two primary processes. Phosphorylation[10] very probably occurs by the energy released between cycles 2 and 3. With reduced triphosphopyridine nucleotide and adenosine triphosphate, carbon dioxide can be reduced to sugar via the Calvin cycle[11]. The reaction pattern is in accordance with the results already reported here and also with the following findings (20° C., $pH \approx 7$).

(1) On excitation of Chl*a*-670 and Chl*a*-680 in *Chlorella*, the life-time τ of the changes in absorption at

420 mμ and 515 mμ are the same ($\tau_{420} = \tau_{515} = 2 \times 10^{-3}$ sec.) because oxidized cytochrome and X react jointly.

(2) On excitation of mainly Chl*a*-680, τ_{420} is greatly prolonged (up to $\tau_{420} \approx 1$ sec.), as only small amounts of X are formed for the reduction of oxidized cytochrome.

(3) In the case of spinach chloroplasts extracted with water, oxidized dyes with a redox potential up to ~ 0 V. can be coupled to X (ref. 4). This is shown by the observation that τ_{515} is greatly shortened (down to $\tau_{515} \approx 10^{-3}$ sec.) as X is oxidized in addition by the dyes.

(4) Vice versa reduced dichlorophenol indophenol (red. In.) can be coupled to Cyt0. In this case τ_{420} is greatly shortened (down to $\tau_{420} \approx 10^{-3}$ sec.) as Cyt0 is reduced additionally by red. In.

(5) Preliminary experiments show that CMU (*p*-chlorophenyl-dimethylurea) uncouples cycle 4 from oxygen production. Heating to 50° C. destroys cycles 3 and 4. Heating to 60° C. destroys cycles 1 and 2. At the same temperature, type 1 chlorophyll reaction ceases[2]. Probably reaction type 1 sensitizes cycles 1 and 2.

The reaction pattern of Fig. 2, derived from experimental observations, is similar to a hypothetical one given by Hill[12].

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Note added in proof. (1) In the meantime the redox potential of cycle 2 has also been estimated experimentally. This was done as in the case of cycle 3 by coupling different substances (red. In., ferrocyanide, etc.) to Cyt0. The result is a potential of $\sim +0{\cdot}4$ V. (2) Prof. H. Kuhn has directed our attention to a publication of Prof. H. Kautsky[13] in which a pattern corresponding with Fig. 2 was derived from changes of fluorescence. The principal differences between both will be discussed in a later publication.

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Effect of Spacing and Nitrogen-level on Floral Initiation in Cocksfoot (*Dactylis glomerata* L.)

Cocksfoot, like many perennial herbage grasses, is a long-day plant[1], and is reported to have an obligate winter requirement for cold and short days[2] or cold alone[3], before floral initiation can occur. It has not been possible to satisfy this requirement artificially by cold or short-day treatment given to germinating seed or young seedlings, (Calder, D. M., and Cooper, J. P., unpublished data) although such treatment has been effective in *Lolium* and the winter cereals[1]. Preliminary observations suggested that spacing of plants and nitrogen supply might have an overriding influence on flowering in cocksfoot and the following experiment was planned to investigate these effects.

Two contrasting varieties, *Bc* 4542, a natural population from Israel, and *S.*143, a late-flowering variety developed at Aberystwyth, were sown outdoors on April 29, 1960. At the third-leaf stage 160 plants of each variety were transplanted into 8-in. pots containing steam-sterilized soil, 80 at the rate of 10 plants per pot and 80 at one plant per pot. These spacing treatments were further divided into high and low nitrogen-levels, the high nitrogen group having Nitro-Chalk added at the rate of 1 gm. per pot every 2 wks., while the low nitrogen-level group had no nitrogen added. There were thus four spacing/nitrogen treatments per variety, each treatment containing ten plants, replicated four times.

Table 1. Frequency of Reproductive Plants in Spacing and Nitrogen Treatments

Variety	One plant/pot		Ten plants/pot	
	High N	Low N	High N	Low N
Bc 4542				
Reproductive	35 (5)*	25 (3)	9 (2)	0 (0)
Vegetative	5	15	31	40
S. 143				
Reproductive	15 (4)	6 (4)	0 (0)	1 (0)
Vegetative	25	34	40	39

Variety, spacing and nitrogen differences all significant at $P < 0{\cdot}001$.
* Figure in parentheses denotes number of plants with heads initiated but not emerged.

Table 1 shows the number of plants in each treatment which produced heads by September 26 (150 days after sowing), the number which initiated inflorescences without ear emergence, and the number remaining vegetative. No further initiation would be likely to occur after this date, because of a decreasing photoperiod. Both spacing and nitrogen-level had a marked influence on floral initiation, although the Israel population produced a higher proportion of flowering plants than *S.*143 in practically all treatments. In the denser spacing with low nitrogen, only one plant out of 80 produced heads, while in the wider spacing with high nitrogen 87·5 per cent of the Israel plants and 37·5 per cent of the *S.*143 initiated heads. Although the level of nitrogen applied has not compensated for the more severe competition at the denser spacing, the spacing/nitrogen interaction is not significant, suggesting that nitrogen supplied at greater rates might overcome the effects of plant density.

Little work has been done on the effects of plant spacing on floral development in herbage grasses and cereals, and reports on the effects of nitrogen-level are somewhat conflicting. In winter rye, for example, Gott *et al.*[4] have shown that nitrogen has little or no effect on any stage of the flowering reaction in fully

vernalized plants, but in unvernalized material a low nitrogen-level may slightly retard progress to flowering. Von Denffer[5], on the other hand, reports that nitrogen deficiency accelerates flowering in barley and wheat. Leopold[6] states that, in general, nitrogen-level may have a profound influence on stages subsequent to floral initiation, but not on initiation itself. In the present case, both spacing and nitrogen-level have had a pronounced effect on floral initiation in cocksfoot. None of the plants received any cold exposure and the photoperiod was more than 13 hr. throughout the experiment, which indicates either that wide spacing and high nitrogen-level have largely replaced the inductive requirement, or that dense spacing with low nitrogen have inhibited the photoperiodic response.

These results should have important implications for the management of cocksfoot swards for continued leaf production on one hand, or for seed production on the other. They also provide a possible explanation for the findings of Evans[7] on the effects of plant density on seed-yield in a number of herbage grasses.

It is a pleasure to acknowledge the facilities and encouragement given by the director, Prof. P. T. Thomas.

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Effects of Light Intensity on Reproduction in *S.*48 Timothy (*Phleum pratense* L.)

In recent years the role of light intensity as a major factor controlling growth of herbage in the sward has been emphasized. The production of inflorescences in herbage grasses also appears to be influenced by light intensity in conditions otherwise known to favour the onset of floral initiation. Several investigators[1–3] have remarked on the reduced flowering obtained in the glasshouse in winter, when photoperiod and temperature are artificially maintained at summer-levels. Experiments in the glasshouse have shown that inflorescence production in *S.*48 timothy occurs readily in the spring and early summer, whereas floral initiation is sporadic and delayed when plants are exposed to similar photoperiods and temperatures in the autumn and winter. In an attempt to separate the effect of light intensity, which presumably exerts an indirect effect on flowering by influencing photosynthesis, from that of factors such as photoperiod, which have a direct effect on flowering, an experiment was conducted in summer to test the effect of reducing the natural light intensity on the reproduction of *S.*48 timothy.

In 1958, seed of timothy was sown on damp vermiculite on March 20. A few days after germination the seedlings were transplanted to 3-in. pots of soil maintained under 8-hr. day-lengths in an unheated glasshouse. After the production of 7–8 leaves, the plants were moved outside to soil beds and planted 5 in. apart. Three of the beds were covered with layers of muslin arranged on metal frames to give varying light transmissions; the fourth bed of plants was left uncovered to provide a control. During the first half of the experiment, integrated light readings under the different treatments were obtained with Megatron photometers; the percentage transmissions in terms of natural light decreased in the following manner: (*A*) control 100 per cent, (*B*) 60 per cent, (*C*) 51 per cent and (*D*) 33 per cent. Thermograph recordings showed reduced temperature differentials under the muslin covers compared with the control, but the mean daily temperatures did not differ substantially.

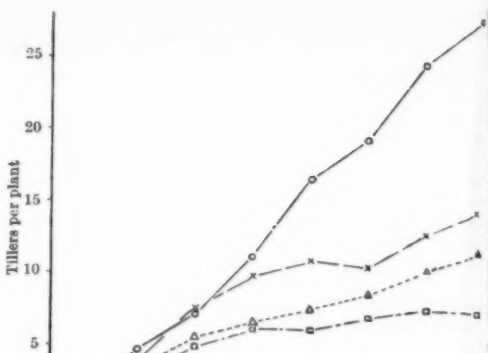

Fig. 1. Influence of light intensity on tillering in *S.*48 timothy. In full daylight, ○; in 60 per cent daylight, ×; in 51 per cent daylight, △; in 33 per cent daylight, □

The influence of light intensity on vegetative growth was assessed from tiller numbers before the ears began to emerge (Fig. 1). Light intensity had a marked effect on tiller numbers, and a significant response began to appear 2–3 weeks after the beginning of the treatments. After 7 weeks the control plants had produced nearly four times as many tillers as comparable plants in the densest shade; and this trend continued to the final harvest (Table 1). Several plants receiving only 33 per cent of the

Table 1. Effects of Light Intensity on Reproduction in *S.*48 Timothy. (Mean of 32 Plants)

Main stem	*A* 100 per cent (full daylight)	*B* 60 per cent	*C* 51 per cent	*D* 33 per cent
Mean No. days to ear-emergence	57·4 ± 0·63	58·0 ± 1·02	59·0 ± 1·05	71·8 ± 2·02
Mean leaf No. to ear-emergence	14·6 ± 0·15	13·9 ± 0·26	14·0 ± 0·23	15·5 ± 0·41
Mean ear-length (cm.)	9·6 ± 0·33	9·2 ± 0·39	8·5 ± 0·34	6·2 ± 0·45
Mean stem-length (cm.)	80·4 ± 1·87	100·1 ± 1·60	101·0 ± 1·52	90·7 ± 1·85
Percentage fertility	100	100	100	67
Whole plant				
Mean tiller No./plant	38·0 ± 2·99	28·4 ± 1·90	24·5 ± 1·86	12·4 ± 0·92
Mean ear No./plant	12·2 ± 1·01	8·4 ± 0·76	6·1 ± 0·52	1·9 ± 0·44
Percentage fertility	32·2	29·6	24·9	15·4

natural light carried only three or four living tillers when the experiment was terminated. The mortality of new tillers was very high in this degree of shading.

Ears began to emerge on the main stems, first on the control and lightly shaded plants and later on the plants in dense shade. The final results for days to emergence of the ear (Table 1) showed increasingly delayed emergence which became significant when the light was reduced from 51 to 33 per cent of full daylight. To some extent, this trend was reflected in the mean leaf numbers to ear-emergence, which also increased in the densest shade, but the leaf number in full daylight was exceptionally high. However, at the lowest level of light, the delayed ear-emergence, in those plants that did flower, was accompanied by a complete suppression of reproduction in about a third of the main stems (Table 1). These shoots showed internode elongation similar to that of flowering shoots, but micro-dissections at the final harvest revealed strictly vegetative apices. Length of ear in the flowering shoots also showed the effect of light intensity. In normal light conditions, ears averaged almost 10 cm., whereas, as the shading was intensified, the length decreased progressively to approximately 6 cm. Unpublished evidence from other experiments has revealed a good linear relation between ear-length and total floret number in this variety; thus a direct relation between light intensity and potential seed yield per shoot is indicated. The mean number of ears per plant decreased to one-sixth as the light intensity fell from full daylight to dense shade, with the result that the percentage of fertile tillers per plant was also markedly influenced.

These results show that reduced light intensity appreciably affects both vegetative and reproductive growth in *S.*48 timothy. The total daily radiation received by plants in the densest shade increased from 80 to more than 100 cal. cm.$^{-2}$ throughout the period of the experiment, an intensity frequently exceeding that found in the glasshouse during autumn and winter. Similarly, the light intensity near ground-level in developing herbage stands falls in spring to less than 20 per cent of full daylight[4,5], which would suggest that a proportion of the shoots may not receive sufficient light for inflorescence initiation. This may in part account for the elongated, sterile tillers which are observed frequently in timothy seed crops.

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Artificial Production of Buds fom the Embryos of *Cuscuta reflexa*

This communication forms part of an extensive programme on the *in vitro* culture of embryos and ovules. The observations are of interest in that the parent embryo produced additional 'adventive' embryos.

Embryos of *Cuscuta reflexa* (0·5–2·5 mm. long) were cultured on a modified White's medium containing minerals + sucrose (5 per cent) + casein hydrolysate (400 mgm./l.) + indole-3-acetic acid (1 mgm./l.).

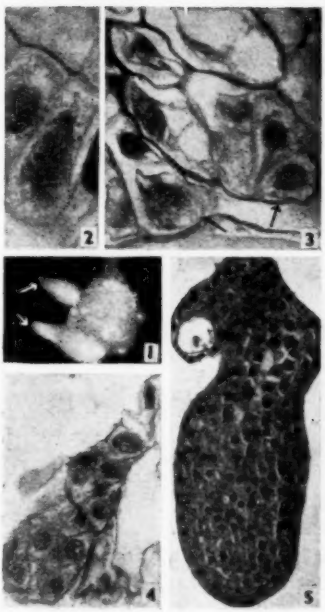

Fig. 1. Fifty-day-old culture of zygotic embryo showing adventive embryos at the radicular end (arrows); note the narrow apical end above. (× *c.* 5·3)

Figs. 2 and 3. Portions enlarged from a transection of the 'radicular' end of zygotic embryo, 40 days after inoculation, showing a dividing cell and two-celled proembryo (arrow-marked) respectively. (× *c.* 233)

Figs. 4 and 5. Further stages in the development of adventive embryos. (Fig. 4, × *c.* 170; Fig. 5, × *c.* 100)

When relatively small embryos (0·5–1·0 mm. long) were taken, they did not pass through the normal stages of development but swelled considerably and ultimately became transformed into a mass of callus. Small, whitish or yellowish-green bodies differentiated from this mass within six weeks of inoculation. They strongly resembled normal embryos. When larger embryos (1·0–2·5 mm. long) were cultured, they produced seedlings within a month. However, callus developed at certain points from the 'radicular' end, and, as in the cultures of smaller embryo, adventive embryos differentiated either from the callus or directly from the superficial cells (Fig. 1). Sometimes 10–15 such embryos were produced in a single culture.

Externally these embryos of adventive origin resembled normal embryos in possessing a narrow apical end and a broader 'radicular' end (Fig. 1). During their development, some of the epidermal cells of the original embryo became large and meristematic and underwent a transverse division (Fig. 2) to form 2-celled proembryos (Fig. 3). Further irregular divisions resulted in a globular structure looking like an embryo (Fig. 4). As in Nature[1], the embryos, instead of passing into a heart-shaped stage, showed vertical elongation (Fig. 5) and ultimately the distal end differentiated into the shoot apex.

The formation of vegetative buds has been previously reported from the callus of tobacco and carrot[2–4] and the embryos of some orchids[5]. Reinert has emphasized the close resemblance of the vegetative buds arising from the callus of carrot tissue with

adventive embryos. In the present work it has been shown that in their development these adventive structures pass through many important stages fully resembling those in the normal development of the embryo.

Some of the adventive embryos proceeded to form a normal shoot, but the majority callused if left in the same medium. This callus is capable of continuous growth and produces a fresh crop of adventive embryos, thus providing an unlimited source of similar material.

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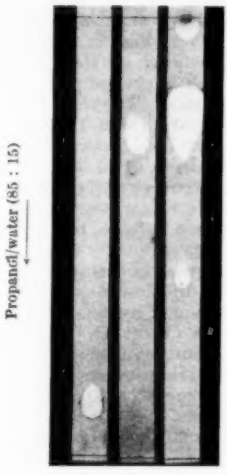

Fig. 1. Paper chromatograms of *a*, NaDDC dissolved in water (0·03 ml. of 33 p.p.m.); *b*, 1 : 1 alcoholic solution of sap from hypocotyls and cotyledons of cucumber seedlings after uptake of NaDDC by the roots (0·03 ml.); *c*, the same solution as in *b* (1·2 ml.)

Transformation in Plants of Sodium Dimethyldithiocarbamate into other Fungitoxic Compounds

In investigations concerning plant chemotherapy it was demonstrated that the fungicide sodium dimethyldithiocarbamate (NaDDC), having been taken up by cucumber seedlings (*Cucumis sativus* L.), has some chemotherapeutic activity against *Cladosporium cucumerinum*[1]. In work on the uptake and translocation of NaDDC by these plants I have found that dimethyldithiocarbamate could not be recovered from the tissues. Instead the presence could be demonstrated of three unknown fungitoxic compounds which have the same mode of action as NaDDC, but differ in chemical properties. These observations show that, in the plant, NaDDC is subject to certain transformations which most probably are of a biochemical nature.

I have found that the addition of plant sap to NaDDC strongly reduces its fungitoxicity. For this reason small amounts of NaDDC in plant sap ($\leqslant$20 p.p.m.) cannot be assessed by a direct determination of the fungitoxicity of the sap. If, however, a solution of NaDDC (10 p.p.m.) in cucumber sap is chromatographed, the presence of a fungicide can be detected with an R_F value of 0·87. To this end the solvent propanol/water (85 : 15) is allowed to run down the paper for 16 hr. at 22° C. The chromatogram is developed according to Weltzien[2], by spraying with a conidial suspension and subsequent incubation. Highly satisfactory chromatograms were obtained by adding an equal volume of ethanol (96 per cent) to the sap and placing 0·03 ml. or a higher quantity of the resulting supernatant on 1 cm. of the starting line of Whatman paper No. 3; the strips were developed by placing them on a metal frame stringed with nylon threads and subsequent spraying with a conidial suspension of *Glomerella cingulata* in a glucose-mineral salts medium (10^7 conidia/ml.). In this medium *G. cingulata* is highly sensitive to dialkyldithiocarbamates[3]. The strips were incubated for two days in a moist glass box at 27° C.

If a chromatogram is made of NaDDC dissolved in water (Fig. 1*a*) instead of in plant sap, a fungicide is detected at the same spot (R_F 0·87). Yet, in neither of these cases does the spot contain NaDDC. Instead the presence of its copper salt, $((CH_3)_2NCS)_2Cu$, could be demonstrated spectrophotometrically by Dr. M. J. Janssen. Apparently, under the present conditions, NaDDC forms a chelate with copper from the paper.

Without previous chromatography the presence of small amounts of NaDDC in plant sap cannot be revealed on paper; this suggests that the sap contains antagonists of NaDDC which are separated from the fungicide by the chromatographic procedure.

Once this method of detecting NaDDC in plant sap had been developed, I chromatographed the sap of cotyledons and hypocotyls of ten cucumber seedlings which had been standing for 2 days with their roots in 50 ml. of a 150 p.p.m. solution of NaDDC. Development of the strip, to our surprise, revealed a fungitoxic compound with an R_F value of 0·27 (Fig. 1*b*) while no fungicide could be detected with an R_F value of 0·87. If, moreover, the sap was concentrated about forty times, two other fungitoxic compounds (R_F values 0·03 and 0·58) were visible as well (Fig. 1*c*).

All three fungicidal compounds have the same mode of action as dimethyldithiocarbamate, since they could be counteracted by the addition of histidine or dibutyldithiocarbamate[4] to the nutrient medium used for spraying the strips. I therefore must assume that the three unknown compounds are derivatives of dimethyldithiocarbamic acid, which most probably have been produced by mediation of plant enzymes.

This transformation of NaDDC into other compounds which exert fungitoxic activity was also found to occur in broad bean plants (*Vicia faba* L.) and in chicory (*Cichorium intybus* L.).

It seems possible that biochemical transformation products of NaDDC in the plant are responsible for the observed chemotherapeutic effect.

I thank Dr. A. Kaars Sijpesteijn for many helpful suggestions. This work was carried out as part of the programme of the T.N.O. Research Unit for Internal Chemotherapy of Plants, at the Laboratory of Phytopathology, Wageningen, The Netherlands.

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ENTOMOLOGY

Induced Changes in the Amounts of Material in the Neurosecretory System of the Desert Locust

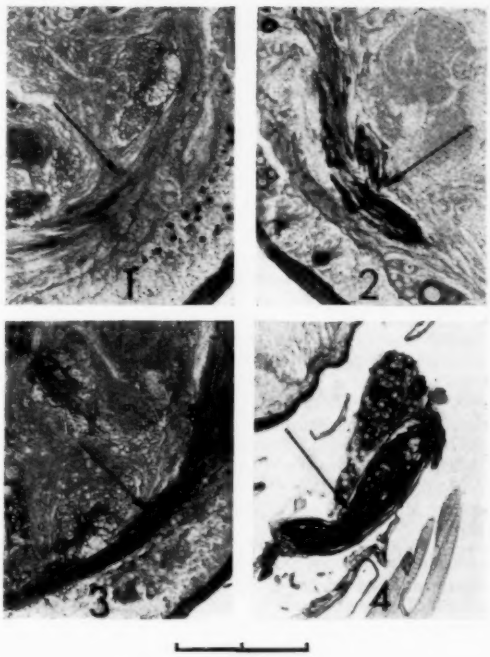
MATURE adult female *Schistocerca gregaria* contain large amounts of neurosecretory material in the corpora cardiaca, the nervi corporis cardiaci I and the neurosecretory cells of the pars intercerebralis of the protocerebrum[1]. The neurosecretory systems of 2-week-old females, reared without males, similarly contain large amounts of stainable material; in these females, the terminal oocytes are largely undeveloped.

Forty-five minutes after administering high-frequency electrical shocks (10 V., 40 pulses/sec. for periods of 15 min. or longer) through the optic nerves of 2-week-old females, reared without males, the neurosecretory systems are almost devoid of stainable material (Fig. 1). Low-frequency electrical shocks (10 V., 40–75 pulses/min. for 15–30 min.) administered through the ventral nerve cord, the surface of the brain or through the optic nerves, result in the appearance of a very large excess of material along the nervi corporis cardiaci I (Fig. 2) and within the storage regions of the corpora cardiaca, compared with the operated controls (Fig. 3).

These effects of high- and low-frequency electrical stimulation are not necessarily inconsistent. It is probable that both kinds of stimulation accelerate movement of neurosecretory material along the nervi corporis cardiaci I and into the corpora cardiaca (and presumably into the blood). Low-frequency stimulation illustrates the early stages of the process; high-frequency stimulation the final stages.

Depletion of material in the neurosecretory systems of 2-week-old females, reared without males, also results when the insects are rotated in a flask for 45 min. in such a way that they are continually turned upside down and continually struggle to regain their normal orientation. Hodgson and Geldiay[2] record a diminution in amount of neurosecretory material in the corpora cardiaca of *Blaberus craniifer* following similar electrical stimulation and enforced activity.

Adult females killed during their first observed copulation reveal amounts of neurosecretory material along the nervi corporis cardiaci I (Fig. 4) and within the corpora cardiaca which are remarkably similar to the amounts along the nerves and in the glands of females after low-frequency electrical stimulation. Twelve hours after copulation, the material in the neurosecretory system is in small amount, resembling the condition following high-frequency electrical stimulation and enforced activity. It is clear that in this respect electrical stimulation and enforced activity mimic the effect of the natural stimulation of copulation.



Figs. 1–4. Photomicrographs of brain sections of *S. gregaria* after various treatments. Heads fixed in Bouin's fluid and sections stained with paraldehyde–fuchsin following permanganate oxidation. Arrows point to the nervi corporis cardiaci I in each figure; neurosecretory material appears black. 1, Nervi corporis cardiaci I in brain of female after 15 min. of high-frequency electrical shocks through the optic nerves; amount of neurosecretory material negligible. 2, Nervi corporis cardiaci I in brain of female after 15 min. of low-frequency electrical shocks through the optic nerves; very large amount of neurosecretory material present. 3, Nervi corporis cardiaci I in brain of female after electrodes had been in position in the optic nerves for 15 min., but the animal not stimulated (operated control); amount of neurosecretory material intermediate between 1 and 2. 4, Nervi corporis cardiaci I just beneath the brain; female killed *in copula*; very large amount of neurosecretory material present

Norris[3] has shown that copulation in *S. gregaria* is normally necessary for the development of eggs to their full size, preceding oviposition. Further, neurosecretory cells are necessary for egg development in this species[1]. The present observations suggest that copulation in *S. gregaria* makes available larger amounts of neurosecretory material for the full development of the eggs.

It should, therefore, be possible to simulate the effect of copulation on egg development by subjecting virgin females to electrical shocks or enforced activity. The results of electrical stimulation are inconclusive, since, although the terminal oocytes develop rapidly following the treatment, so also do the terminal oocytes of the operated controls. It is known that injury can accelerate maturation in the desert locust[3]. Enforced activity in 14-day-old females reared without males produces changes in the amount of material in the neurosecretory system similar to electrical stimulation, but the insects are not injured in the process. Fourteen 14-day-old females, reared without males, were subjected to enforced activity for 1 hr./day for 3 days. Dissected seven days later, the

terminal oocytes measured 5·56 ± 0·64 mm.; 10 control females placed in the flask but not rotated possessed terminal oocytes 2·69 ± 0·57 mm. long seven days later. This result supports the hypothesis that the availability of neurosecretory material is important for egg development.

Hodgson and Geldiay[2] have suggested that the loss of adrenalin-like activity of extracts of corpora cardiaca of *Blaberus craniifer* following electrical stimulation or enforced activity should be correlated with the disappearance of neurosecretory material from the glands. It is difficult to reconcile this view with Cameron's observation[4] that the corpora cardiaca of *Periplaneta americana* yielded extracts with adrenalin-like activity, even after the nervi corporis cardiaci had been cut some time previously. The present results suggest that the release of neurosecretory material from the corpora cardiaca of *S. gregaria* has a positive effect on oocyte development; experiments in progress indicate that an adrenalin-like substance is produced from the *glandular* parts of the corpora cardiaca in *S. gregaria*.

I am grateful to the Anti-Locust Research Centre, London, for providing the locusts used in this work; and to Mr. W. Mosley for making the photomicrographs.

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PSYCHIATRY

Spike and Wave Discharges and Alterations of Conscious Awareness

During a study of the mental concomitants of electroencephalographic changes in human beings suffering from petit mal, it was observed that changes in level of awareness did not coincide with the onset and cessation of surface spike and wave activity.

The minimal clinical signs of petit mal attacks are usually accompanied by the most obvious and dramatic electroencephalographic evidence of surface electrical changes, yet no other evidence of pathology in the nervous system has so far been discovered. The clinical accounts of the condition include all degrees of interruptions of conscious awareness with variations in form and frequency of the spike and wave activity. Our work was restricted to patients exhibiting typical clinical petit mal, with the classical bilaterally synchronous 3/sec. spike and wave activity seen on the electroencephalogram usually lasting 3–30 sec.

Eight patients were examined during the simultaneous recording of the electroencephalogram, certain peripheral autonomic changes and the interview. In all, 1,075 petit mal attacks were clinically observed. Five cases were recorded for a total of 31¼ hr. during which 91 attacks were recorded: in addition 3 cases were studied intensively for a total of 97½ hr. during which 684 attacks were recorded, and this latter group formed the main material for this work. Particular attention was paid to alterations in conscious awareness correlating with episodes of spike and wave activity of more than 3 sec. duration. The investigation of mental state was by question and answer, so that the highest level of brain function was involved since an appreciation of meaning was necessary for a sensible answer.

The onset of spike and wave activity varied greatly as to amplitude, lateral predominance and time of recruitment to full amplitude (Fig. 1).

During period *A*, when the spike and wave activity had reached full amplitude, the patient was capable of speaking and answering questions, and during or following period *D* could similarly recall events; the cessation of speech did not coincide consistently with any change in the electrical records.

Questions given in period *C* could produce the response 'Mmmm ?' during this period, or the answer to the question during or following period *D*. Any movement by the patient during this period could be recalled after the attack.

In period *D*, when spike and wave activity had ceased, there was a variable delay before reply, but on other occasions an immediate response was forthcoming. There was often an increase in pulse-rate about 1½ sec. after the end of spike and wave activity.

In period *B*, the patients were unable to respond to questions or to recall afterwards events during it. They were capable of a variety of movements and changes in facial expression (for example, smiling), and very frequently in two patients it was noticeable that, although they often turned their heads to one side, they could still return their gaze to look at the observer as spike and wave continued even when he changed his usual position from one side of the bed to the other before or after the attack had started.

It appeared from the above that the patients could speak for 3 sec. after the onset of an attack. They could perceive speech for 1½ sec. before the end of spike and wave activity, since they could answer the question or on occasion reply 'Mmmm ?' after the end of the attack. It also appeared that the ability to speak was sometimes delayed for some seconds after the spike and wave activity had ceased. It was observed that changes in expression of the eyes occurred before and after the end of spike and wave activity.

Alterations in pulse-rate, psychogalvanic skin response and respiration rarely correlated during the incidence of spike and wave activity. In one patient changes in pulse-rate were clearly seen; occasionally there was an increase in rate which usually occurred about 1½ sec. after the end of spike and wave; but it could also occur some seconds before or after the end of the attack. Changes before the onset of the attack were inconsistent and were complicated by sinus arrhythmia.

In other circumstances it has been demonstrated that electro-physiological changes may not be coincident with changes in conscious awareness[1,2]. Bates[3] also has demonstrated that patients can talk up to 3 sec. after the onset of electroencephalographic spike

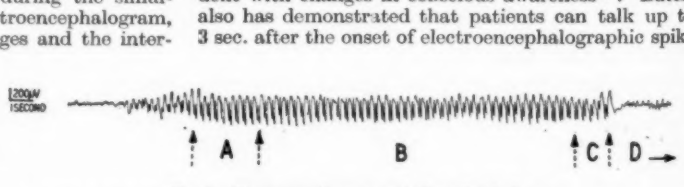

Fig. 1. Diagrammatic representation of attack.
Interval *A*, first 3 sec. of full-amplitude bilaterally synchronous spike and wave activity; *B*, duration of the attack excluding the last 1½ sec. of spike and wave that occur before any final slow wave; *C*, last 1½ sec. of spike and wave before any final slow wave; *D*, end of spike and wave and an arbitrary 10-sec. period

and wave activity; A. Jus (personal communication) using different methods has found that the cessation of spike and wave pattern does not coincide with the return to consciousness. Since the attempt in animal experiments is to simulate the clinical characteristics of human petit mal, it is important, as Jasper[4] has also urged, that a close examination be made of them. It may be worth while to study complex changes in psychological function in circumstances such as at operation when electrocorticograms and depth recordings are made.

These observations lead one to think of a common underlying process which variously affects consciousness and the cerebral electrical activity as recorded from the scalp. It has been shown that more refined techniques are necessary for the investigation and study of subtle alterations of conscious awareness. The causal connexion between spike and wave activity and alterations in consciousness deserves special investigation, since it appears that gross electroencephalographic changes are related to mental changes but that the points in time at which these events occur do not correlate.

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* Supported by a Ford Foundation Grant.

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Distribution of Piperidine in the Brain and its Possible Significance in Behaviour

RECENT work on the use of piperidine for treating psychiatric disorders[1] has prompted an extensive programme here to investigate the mechanism of action of this simple chemical. The basis for using piperidine as a psychotherapeutic agent originally developed from the discovery that the agent antagonized the central actions of N-methyl 3-piperidyl-glycolate and other piperidinoglycolates possessing psychotomimetic properties[2]. These findings were particularly significant because piperidine has been found in human urine[3] and mammalian brains[4]. Piperidine might be an endogenous agent serving some normal function in the regulation of behaviour; that is, perhaps the amine is an endogenous psychotropic agent.

How much piperidine actually enters the brain after the agent is administered in an effective dose would be significant. Experiments were therefore conducted in rabbits, using tritium-labelled piperidine. The material was made by reducing pyridine with tritium, using palladium as a catalyst. After repeated recrystallization of the hydrochloride salt, the final material had a specific activity of 285 μc./mgm. Adult male albino rabbits were injected intravenously with 100 mgm./kgm. of piperidine hydrochloride per kgm. body-weight. This dose produced marked muscle relaxation and sedation in rabbits. After 30 min., the rabbits were bled by carotid cannulation, and the brain was perfused with saline until all trace of jugular blood was removed. The brain was carefully removed and dissected. After being weighed in the frozen state, the brain segments were suspended in 20 volumes of 5 per cent formalin for a period of 24 hr. at room temperature, a procedure extracting over 99 per cent of the piperidine (the formalin extract was filtered and dried in an oven at 90°). Further preparation of the dried residue for counting in a liquid scintillation counter is described in ref. 5.

The greatest concentration of piperidine occurred in the caudate nucleus while other regions in the diencephalon contained much less than half the concentration of the caudate (Table 1). Within the telencephalon, the greatest concentration appeared in the anterior neocortex, and although the lowest concentration occurred in the white matter, the concentration in the corpus callosum was relatively high. Next to the caudate, the quadrigemmina contained the greatest concentration of piperidine. Distribution of piperidine into the various segments of the diencephalon and mesencephalon was fairly uniform. With the exception of the telencephalon, the distribution of piperidine between grey and white matter was surprisingly similar.

At present, it is not possible to relate the distribution of piperidine to its action on the central nervous system, although a few general remarks can be made. Preliminary findings indicate that the electrophysiological effects of the piperidinoglycolates in regions such as the reticular formation and caudate nucleus can be reversed by piperidine, which is a weak nicotinic agent. It remains to be seen whether the muscle relaxation and weakness, ataxia, and the generalized decrease in motor responsiveness produced by piperidine in rabbits can be attributed to the distribution of the drug in such regions as the anterior neocortex and caudate nucleus.

In view of the fact that piperidine is normally present in mammalian brain, the question arises as to its possible significance in normal brain function. Honegger and Honegger[4] have reported the concentration of about 0·05 μgm. of piperidine per gm. of rabbit brain, which is approximately one-tenth the concentration present when 100 mgm./kgm. of the drug is administered (Table 1). When one considers that piperidine normally may be localized in certain brain regions, and that it appears to be excreted rapidly in quite large amounts (20 mgm./day in

Table 1

Vesicle	Derivative	Counts/mgm.
Telencephalon	anterior neocortex	6·6
	posterior neocortex	5·3
	pyriform cortex	3·5
	hippocampus	3·1
	white matter	1·8
	corpus callosum	3·2
Diencephalon	corpus striatum	2·5
	caudate nucleus	10·8
	lateral thalamus	1·7
	medial thalamus	3·1
	hypothalamus	4·8
Mesencephalon	tegmentum	3·3
	foot of mid-brain	3·2
Rhombencephalon	cerebellum	3·8
	pons	3·5
	medulla	3·6
	quadrigemmina	7·5

Localization of tritium-labelled piperidine in various regions of rabbit brain. Radioactivity is expressed in terms of counts/min./mgm. wet weight tissue. Since the ratio unlabelled piperidine/tritiated piperidine = 100, and since the specific activity of the tritiated material is 285 μc./mgm., 1 μgm. of piperidine is equal to approximately 3,000 c.p.m. The total amount of piperidine in a rabbit brain weighing 8 gm. would then be approximately 5 μgm. 30 min. after injection.

human beings)[3], it is quite conceivable that the endogenous piperidine may be influencing behaviour. Von Euler[3] had speculated that, because of its nicotinic property, piperidine may be regarded as an endogenous 'synaptotropic substance'. An important problem is to determine whether piperidine is synthesized within the body or arises from some exogenous source. In plants, piperidine and other cycloalkylamines are derived from amino-acids such as lysine, ornithine and proline[6]; but there is no evidence that such biosynthetic pathways can occur in mammalian systems. The presence of piperidine and other volatile amines in the central nervous system[4] nevertheless suggests the existence of many endogenous agents capable of influencing or regulating neural function. One exogenous source of piperidine is directly through the intestinal flora which can decarboxylate lysine to cadaverine, while the latter product is de-aminated and cyclized to form piperidine.

This work was supported by grants from the Mental Health Fund, State of Illinois, and the Teagle Foundation.

We are indebted to Abbott Laboratories for preparing the tritiated piperidine.

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STATISTICS

Nodal Analysis of Associated Populations

A method of subdividing an associated population the individuals of which are specified by the presence or absence of a number of attributes has been described previously[1]; the process is ultimately derived from the *R*-techniques of factor analysis, and the groups of individuals so obtained will be called *R*-groups. The attributes may similarly be subdivided into *Q*-groups by the corresponding *Q*-techniques. There results a 2-way table in which every attribute-individual record is referred to one of each kind of group. The requirement is to extract from this table the most concentrated groups of records, the attribute-individual noda around which the population may be regarded as varying. The problem is essentially that of establishing coincidences between *R* and *Q* factors; even in normal factor analysis the coincidences are impaired in the formation of the correlation matrix[2,3], and in a subdivision process involving successive correlation matrices the divergence may well be considerable.

Despite many attempts, we have found no way of operating directly on the 2-way table, and have therefore devised a method which involves finding the best possible coincidences with each direction taken as reference direction in turn, followed by collation of the two sets of results so obtained. The population is first *R*-divided, producing a group of individuals defined by the possession of the attribute a with the highest loading on the first centroid *R*-axis. The same population is then *Q*-divided; that individual (i') which, of those possessing a, has the highest loading on the first centroid *Q*-axis is used to delimit the group in the other direction. The residual group is similarly delimited by the individual with the highest loading among those not possessing a. Each resulting group we call an *RQ*-group. The division is continued to the significance-level required, resulting in a series of *R*-groups, with each of which is associated its corresponding *RQ*-group. It will normally be desirable to set a lowest acceptable limit for the loading of i'; if no individual attains this level, *RQ* is set $\equiv R$. The transpose of the entire process similarly gives a series of *Q*-groups together with their corresponding *QR*-groups.

Collation consists in taking one group from each side and subjecting the pair to a logical 'and' operation, whereby only those records common to both tables are retained. The conditions for collation are obtained from the singly defined (*Q*- and *R*-) tables. If any *R*-individual possesses all the *Q*-attributes and any *Q*-attribute occurs in all the *R*-individuals, *QR* is collated with *RQ*; the result is a nodum. If any *R*-individual possesses all the *Q*-attributes, but no *Q*-attribute occurs in all the *R*-individuals, *QR* is collated with *R*; the result is an *R*-subnodum. The transposed situation will give a *Q*-subnodum. If neither condition is fulfilled, all records common to *Q* and *R* are discarded. In the unlikely, but perhaps not impossible, case where collation is permissible but the nodum vanishes, the coincidence may be treated as intersecting subnoda.

The noda are extremely concentrated, and contain all the genuinely central information at the significance-level in use. They are not, however, necessarily all the same type. All are defined in the form ($a'i'$); but a nodum may also contain a and/or i (the attributes and individuals which define the non-residual *R* and *Q* groups), and the presence of these may be regarded as conferring a rise in status on the nodum. The subnoda constitute a penumbra of diffuse groups, some at least of which would become noda were the subdivision to be carried further on one side or the other by lowering the significance-level; they are always defined as (a') or (i'), but their status can rise to (a' i) or (i' a).

Our existing *Pegasus* subdivision-programme limits the analysis to a 76 × 76 population matrix. As yet, we have applied the method only to ecological data, but with very promising results. Whereas our earlier *R*-subdivisions[4,5] gave only groupings of quadrats on species-attributes, the 2-way tables provide a double abstraction in terms of both plant and habitat: the doubly defined noda thus represent true 'vegetation-units' in the widest sense.

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FORTHCOMING EVENTS

Monday, July 10—Friday, July 14

INTERNATIONAL COMMITTEE OF DIETICIANS, and the BRITISH DIETETIC ASSOCIATION (at Church House, Westminster, London, S.W.1)—Third International Congress of Dietetics. Theme: "Tradition, Science and Practice in Dietetics".

Tuesday, July 11—Thursday, July 13

ASSOCIATION FOR THE STUDY OF ANIMAL BEHAVIOUR (in the Department of Zoology, University of Cambridge), at 10 a.m. daily—Symposium on "Orientation and Migration"; and Annual General Meeting.

Saturday, July 15—Sunday, July 16

BRITISH ACADEMY OF FORENSIC SCIENCES (at the Londoner Hotel, Welbeck Street, London, W.1)—Meeting on "Crime-Responsibility, Investigation and Treatment". (Second Annual Scientific Meeting.)

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

PRINCIPAL LECTURER (preferably with teaching and industrial experience) IN THE DEPARTMENT OF MATHEMATICS—The Registrar, Welsh College of Advanced Technology, Cathays Park, Cardiff (July 15).

LECTURER or ASSISTANT LECTURER IN MATHEMATICS at the University College of the West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (July 17).

POSTDOCTORAL FELLOW IN ORGANIC CHEMISTRY, to carry out research in the carotenoid field in collaboration with Prof. B. C. L. Weedon—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (July 17).

SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER (with a first- or second-class honours degree in engineering or equivalent, and preferably an interest in stress analysis or the theory of elasticity), for research on wood machining processes and fundamental studies of the theory of cutting—Forest Products Research Laboratory, D.S.I.R., Princes Risborough, Aylesbury, Bucks, quoting Ref. FPRL 3W (July 17).

ASSISTANT BIOCHEMIST (with a degree or equivalent qualification in chemistry, and experience in, or wishing to take up clinical biochemistry)—The Secretary, Welsh National School of Medicine (University of Wales), 34 Newport Road, Cardiff (July 22).

ASSISTANT LIBRARIAN (Grade B) (with a good honours degree) in the University Library, to be Sub-Librarian for the Faculties of Medicine and Veterinary Science—The Registrar, The University, Liverpool, quoting Ref. CV/N (July 22).

RESEARCH ASSISTANT (graduate in botany or graduating this year) IN THE DEPARTMENT OF BOTANY, to assist the Professor in the establishment and maintenance of a collection of plant organ and tissue cultures and in research involving these cultures—The Registrar, University College, Singleton Park, Swansea (July 22).

RESEARCH FELLOWS and RESEARCH ASSISTANTS (with a good honours degree or equivalent in engineering, mathematics, physics or metallurgy) IN THE DEPARTMENT OF AERONAUTICS AND ASTRONAUTICS—The Secretary and Registrar, The University, Southampton (July 22).

ASSISTANT LECTURER IN THEORETICAL PHYSICS—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (July 24).

RESEARCH ASSISTANT (with a good degree in either physics with geology or geology with physics) IN THE SEDIMENTARY RESEARCH LABORATORY, to assist in establishing and operating an X-ray unit, and to undertake research on the application of X-ray techniques to the mineralogy of sediments—The Bursar, The University, Reading (July 24).

LECTURER IN MECHANICAL ENGINEERING—The Registrar, The University, Sheffield (July 29).

AGRICULTURAL CHEMIST, Scientific Officer or Senior Scientific Officer grade (with an honours degree and experience in research on soils and fertilizers), at Broom's Barn Experimental Station, Barrow, West Suffolk, to work on manuring of sugar beet—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (July 31).

LECTURER or ASSISTANT LECTURER IN ORGANIC CHEMISTRY—The Registrar, The University, Manchester 13 (July 31).

LECTURER (preferably with an interest in plant ecology) IN BOTANY at the University of Natal, Pietermaritzburg, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa and London, July 31).

LECTURERS or ASSISTANT LECTURERS (3) (with a recognized honours degree or equivalent qualification, and preferably with experience in teaching and/or research) IN THE DEPARTMENT OF MATHEMATICS, University of Malaya, Kuala Lumpur—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Malaya and London, July 31).

POSTGRADUATE SCHOLAR (suitably qualified in zoology or physiology) at the Gatty Marine Laboratory, St. Andrews, for work on the analysis of nervous systems of lower animals—Joint Clerk to the University Court, College Gate, St. Andrews (July 31).

SENIOR LECTURER or LECTURER (veterinary surgeon) IN VETERINARY MEDICINE, for duty at the field station near Potters Bar, Middlesex—The Secretary, Royal Veterinary College (University of London), Royal College Street, London, N.W.1 (July 31).

LECTURER/SENIOR LECTURER IN CLASSICAL ARCHÆOLOGY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, August 11).

CHAIRS (2) OF MATHEMATICS at the University of Cape Town, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa and London, August 14).

CHAIR OF PHYSIOLOGY in the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, August 18).

READER IN ELECTRIC TRACTION at Imperial College—The Academic Registrar, University of London, Senate House, London, W.C.1 (August 21).

LECTURER (with an honours degree in zoology and preferably qualified to take courses in vertebrate embryology) IN ZOOLOGY at the University of Canterbury, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, August 30).

LECTURER IN PHILOSOPHY at the University of Canterbury, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, August 31).

WAITE CHAIR OF ENTOMOLOGY in the University of Adelaide, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, September 15).

LECTURER or ASSISTANT LECTURER IN PSYCHOLOGY at the University of Canterbury, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, September 30).

ASSISTANT (Grade B) IN THE DEPARTMENT OF MATHEMATICS—The Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S.W.3.

ASSISTANT (man or woman) TO TEACH MATHEMATICS AND SCIENCE up to, and including, "O" level G.C.E.—The Headmaster, Cheney School, Cheney Lane, Headington, Oxford.

DEMONSTRATOR IN PHYSICS—The Registrar, The University, Nottingham.

LECTURER IN BIOCHEMISTRY IN THE DEPARTMENT OF APPLIED MICROBIOLOGY AND BIOLOGY—The Secretary, Royal College of Science and Technology, Glasgow.

LECTURER (well qualified and experienced in any branch of electrical engineering or related branches of physics, and preferably an interest in materials) IN ELECTRICAL ENGINEERING—The Assistant Registrar (Science), The University, Birmingham.

LECTURER (with doctor's degree) IN CHEMISTRY, to teach up to master's degree (lecturing in French)—Rector L. Gillon, University Lovanium, B.P. 121, Leopoldville XI, Republique du Congo.

LECTURER (with doctor's degree) IN MATHEMATICS, to teach up to master's degree (lecturing in French)—Rector L. Gillon, University Lovanium, B.P. 121, Leopoldville XI, Republique du Congo.

RESEARCH ASSISTANT (with, or expecting shortly to have, a first- or good second-class honours B.Sc. or equivalent, for example, A.R.I.C.) IN THE DEPARTMENT OF CHEMISTRY, for electro-chemical studies in molten salts—The Bursar, The University, Reading.

SENIOR RESEARCH ASSISTANT (preferably with research experience in the physics of discharges or allied fields of gaseous electronics), for work on the production and utilization of augmented ion concentrations in flame plasma—Dr. F. J. Weinberg, Chemical Engineering Department, Imperial College of Science and Technology, London, S.W.7.

TECHNICAL ASSISTANT (with practical experience in pot mending, metal cleaning, developing and enlarging of film and/or archæological draughtsmanship) IN THE DEPARTMENT OF ARCHÆOLOGY AND NICHOLSON MUSEUM—The Registrar, University of Sydney, Sydney, New South Wales, Australia.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Arthur D. Little Research Institute. Annual Report for 1960. Pp. 44. (Inveresk, Midlothian: Arthur D. Little Research Institute, 1961.) [15

Department of Scientific and Industrial Research: Geological Survey and Museum. British Regional Geology—North Wales. By Dr. Bernard Smith and Dr. T. Neville George. Third edition. Completely revised by Dr. T. Neville George. Pp. vi+96+12 plates. (London: H.M. Stationery Office, 1961.) 6*s.* net. [15

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